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New 1,11-dimethyl-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradecane-4,8,12-trione derivative as an allosteric modulator of the glutamatergic system

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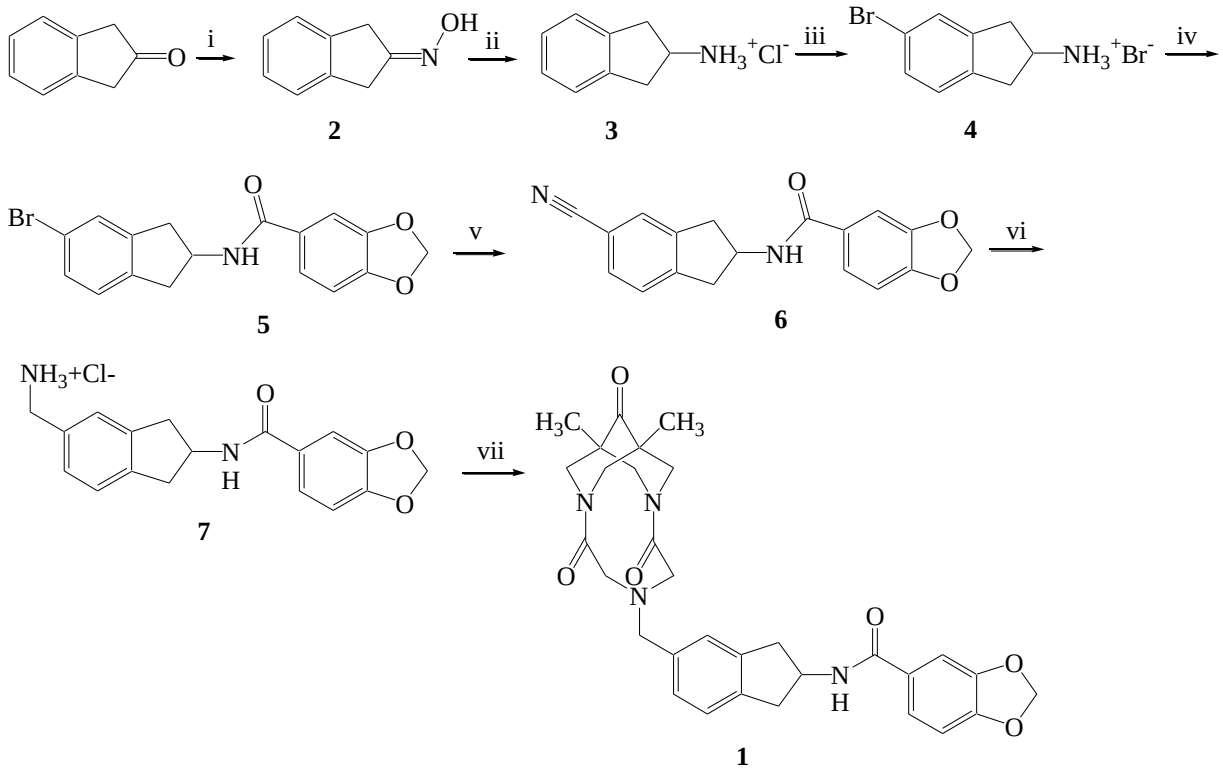
Table of contents

1. Chemistry



Scheme S1 *Reagents and conditions*: i, $NH_2OH{\cdot}HCl$, EtOH, H_2O; ii, H_2, Pd/C, HCl, MeOH; iii, 1) Br_2, H_2O, 2) HBr; iv, 1) KOH, H_2O, 2) piperonylic acid, CDI, MeCN; v, $Zn(CN)_2$, $Pd(OAc)_2$, dppf, NMP/H_2O, PMHS; vi, H_2, Pd/C, HCl, EtOH; vii, 1) KOH, H_2O, 2) 3,7-bis(chloroacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one (**8**), K_2CO_3, NMP.

NMR spectra were recorded on the Agilent 400-MR spectrometer (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts (δ) were measured with reference to the solvents: $CDCl_3$ for ^{1}H (δ = 7.26 ppm), ^{13}C (δ = 77.16 ppm), DMSO-d6 for ^{1}H (δ = 2.50 ppm), ^{13}C

(δ = 39.50 ppm) and CD_3OD for ^1H (δ = 3.31 ppm), ^{13}C (δ = 49.00 ppm). Chemical shifts (δ) are given in ppm; *J* values are given in Hz. When necessary, assignments of signals in NMR spectra were made using 2D techniques. Accurate mass measurements (HRMS) were performed on a Bruker micrOTOF II instrument using electrospray ionization (ESI). The measurements were done in a positive ion mode (interface capillary voltage 4500 V). Analytical thin layer chromatography was carried out with Merck Silica Gel 60 plates (supported on aluminum); the detection was done by UV lamp (254 and 365 nm) and chemical staining (solution of ninhydrin in EtOH). Column chromatography was performed on Merck Silica Gel 60.

*Indan-2-one oxime (**2**).* A mixture of indan-2-one (26.42 g, 0.200 mol) and NH_2OH•HCl (17.32 g, 0.250 mol) in EtOH/H_2O (1:1, 1000 ml) was stirred first for 1.5 h at room temperature and then at 50 °C for additional 1 h. The reaction mixture was cooled to room temperature. The precipitate formed was filtered off and washed with distilled water. The product was recrystallized from EtOH (150 ml). This yielded 20.89 g (0.141 mol, 71%) of pure indan-2-one oxime **2** as needle crystals. mp: 150–153ºC,[S1] spectral data.[S2]

*Indan-2-ylamine hydrochloride (**3**).* A mixture of indan-2-one oxime **2** (5.40 g, 0.037 mol) and 10% Pd/C (3.62 g, 9 mol. %) in 1M HCl/$MeOH_{abs}$ (110 ml) was placed into a glass autoclave and hydrogen was passed through (p=5 atm) while stirring for 6 hours. The reaction mixture was filtered through celite, washed with MeOH, and the solvent was distilled off. This yielded 5.64 g (0.033 mol, 91%) of pure indan-2-ylamine hydrochloride **3** as a white crystalline product. mp: 244–245ºC,[S1] spectral data.[S3]

*5-Bromoindan-2-ylamine hydrobromide (**4**).* To a solution of indan-2-ylamine hydrochloride **3** (2.82 g, 16.6 mmol) in distilled water (12.6 ml), bromine (940 μL, 18.3 mmol) was added dropwise while stirring. The mixture was stirred for 9 h. After that, HBr_{conc} (ω=48%, 2.82 ml, 24.9 mmol) was added, and the mixture was stirred for additional 3 hours. The precipitate formed was filtered off and washed with distilled water (4.5 ml). The product was dissolved in EtOH (37 ml), precipitated with Et_2O and filtered off. This yielded 3.15 g (13.3 mmol, 65%) of pure 5-bromoindan-2-ylamine hydrobromide **4** as a crystalline product. mp: > 300ºC,[S4] spectral data.[S4]

N-*(5-Bromoindan-2-yl)-1,3-benzodioxole-5-carboxamide (**5**).* A mixture of 1,3-benzodioxole-5-carboxylic acid (256 mg, 1.54 mmol) and carbonyldiimidazole (262 mg, 1.62 mmol) in absolute acetonitrile (25 ml) was stirred for 6 hours. In parallel, a 20% aqueous solution of KOH was added to a suspension of 5-bromoindan-2-ylamine hydrobromide **4** (450 mg, 1.54 mmol) in distilled water (pH 10). The resulting mixture was extracted with CH_2Cl_2, the combined organic phases were dried over anhydrous Na_2SO_4, then filtered and the solvent was distilled off. The residue was dissolved in absolute acetonitrile (5 ml), the resulting solution was added to the first mixture, and this was stirred for 24 hours. The solvent was distilled off, and the product was recrystallized from acetonitrile (5 ml). This yielded 388 mg (1.08 mmol, 70%) of pure *N*-(5-bromoindan-2-yl)-1,3-benzodioxole-5-carboxamide **5** as a white crystalline product. mp: 192–193ºC. ^1H NMR ($CDCl_3$), δ: 2.82–2.92 (m, 2H), 3.30–3.41 (m, 2H), 4.89 (m, 1H), 6.01 (s, 2H), 6.22 (d, 1H, J 7.3 Hz), 6.79 (d, 1H, J 8.5 Hz), 7.11 (d, 1H, J 8.0 Hz), 7.22–7.25 (m, 2H), 7.32 (d, 1H, J 8.0 Hz), 7.39 (s, 1H). ^{13}C NMR ($CDCl_3$/CD_3OD), δ: 38.63, 38.98, 50.82, 101.25, 107.11, 107.47, 119.85, 121.48, 125.73, 127.35, 127.73, 129.27, 139.54, 142.98, 147.41, 149.97, 167.10. HRMS (ESI), m/z 359.0153 (calc. $C_{17}H_{14}BrNO_3$ $[M+H]^+$, m/z: 359.0157).

N-*(5-Cyanoindan-2-yl)-1,3-benzodioxole-5-carboxamide (**6**).* A mixture of *N*-(5-bromoindan-2-yl)-1,3-benzodioxole-5-carboxamide **5** (388 mg, 1.08 mmol), $Pd(OAc)_2$ (4.8 mg, 0.021 mmol, 2 mol. %) and dppf (12.0 mg, 0.021 mmol, 2 mol. %) in NMP/H_2O (10:1 by volume, 1.2 ml) was stirred for 1 min. Then PMHS (36 mg) and $Zn(CN)_2$ (65 mg, 0.554 mmol) were added, and the mixture was stirred at 120°C for 12 h. After that the mixture was cooled to room temperature, and

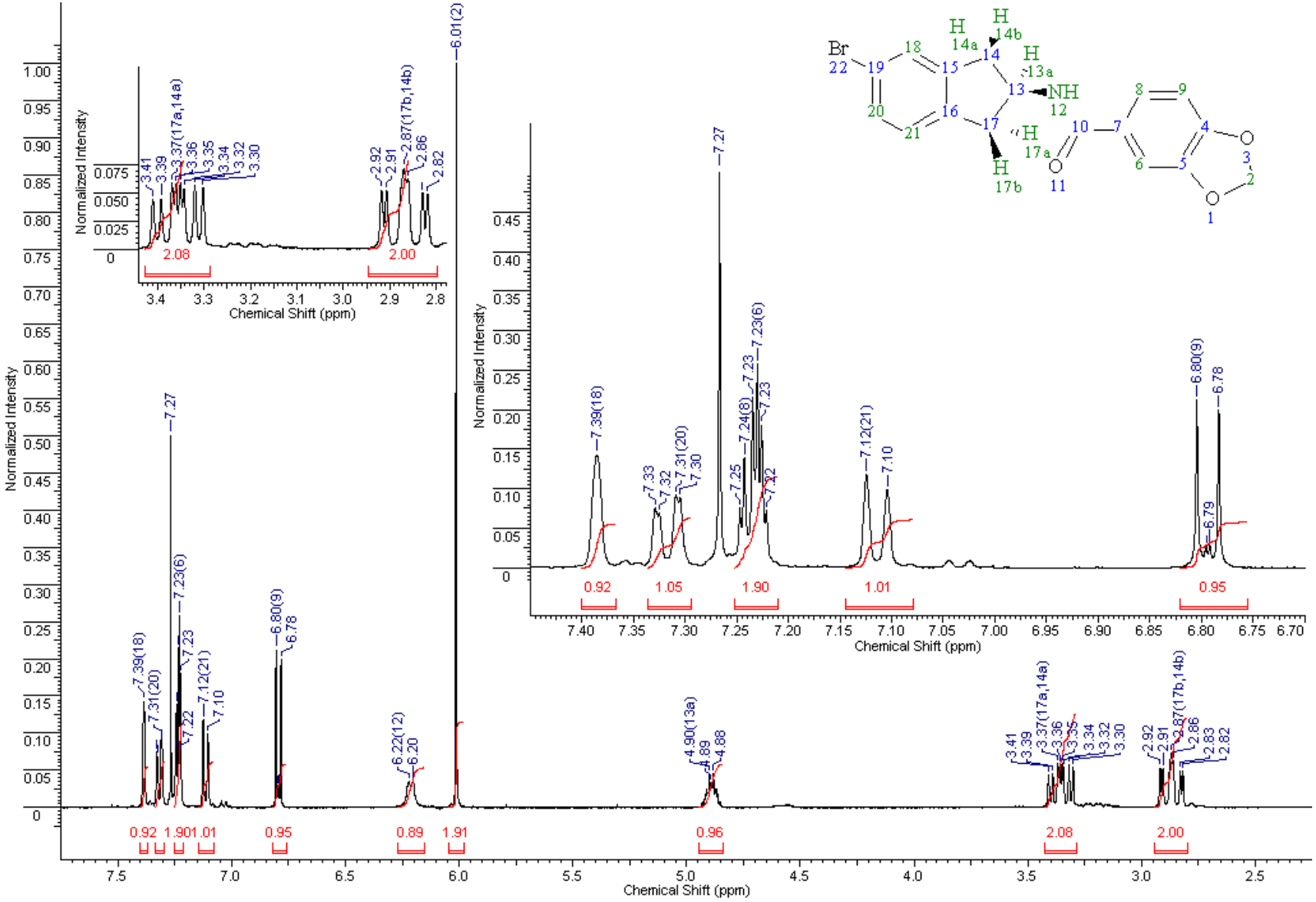
the solvent was distilled off. The resulting product was purified by flash chromatography (eluents CH_2Cl_2, CH_2Cl_2/light petroleum ether (1:1)). This yielded 238 mg (0.78 mmol, 72%) of pure *N*-(5-cyanoindan-2-yl)-1,3-benzodioxole-5-carboxamide **6** as a white crystalline product, mp: 187–188°C. ^{1}H NMR ($CDCl_3$), δ: 2.94–3.02 (m, 2H), 3.40–3.48 (m, 2H), 4.93 (m, 1H), 6.02 (s, 2H), 6.25 (br. d, 1H, J 7.0 Hz), 6.80 (d, 1H, J 7.8 Hz), 7.24–7.26 (m, 2H), 7.34 (d, 1H, J 7.8 Hz), 7.49 (d, 1H, J 7.8 Hz), 7.52 (s, 1H). ^{13}C NMR ($CDCl_3$), δ: 39.65, 40.34, 50.93, 101.73, 107.59, 107.94, 110.59, 119.20, 121.59, 125.63, 128.34, 131.06, 142.30, 146.83, 147.97, 150.45, 166.68. HRMS (ESI), m/z 307.1069 (calc. $C_{18}H_{14}N_2O_3$ $[M+H]^+$, m/z: 307.1077).

N-*[5-(Aminomethyl)indan-2-yl]-1,3-benzodioxole-5-carboxamide hydrochloride (7).* A mixture of *N*-(5-cyanoindan-2-yl)-1,3-benzodioxole-5-carboxamide **6** (110 mg, 0.359 mmol), 10% Pd/C (22 mg, 6 mol. %) and HCl_{conc} (ω=35%, 80 μL) in EtOH (3 ml) was placed into a glass autoclave and hydrogen was passed through (p=5 atm) while stirring for 24 h. The reaction mixture was filtered through celite, washed with EtOH, and the solvent was distilled off. This yielded 116 mg (0.334 mmol, 93%) of pure *N*-(5-(aminomethyl)indan-2-yl)-1,3-benzodioxole-5-carboxamide hydrochloride **7** as a white crystalline product. mp: 239–241ºC. ^{1}H NMR (CD_3OD), δ: 3.01 (dd, 2H, ^{2}J 15.9 Hz, ^{3}J 7.3 Hz), 3.35 (dd, 2H, ^{2}J 16.3 Hz, ^{3}J 8.4 Hz), 4.08 (s, 2H), 4.78 (m, 1H), 6.02 (s, 2H), 6.87 (d, 1H, J 8.1 Hz), 7.25 (d, 1H, J 8.2 Hz), 7.31 (m, 3H), 7.41 (dd, 1H, ^{3}J 8.1 Hz, ^{4}J 1.7 Hz). ^{13}C NMR (DMSO-d6), δ: 42.07, 50.82, 50.94, 101.72, 107.47, 107.80, 122.41, 124.55, 125.14, 126.41, 127.37, 128.33, 132.15, 141.76, 147.24, 149.62, 165.26. HRMS (ESI), m/z 311.1392 (calc. $C_{18}H_{18}N_2O_3$ $[M+H]^+$, m/z: 311.1390).

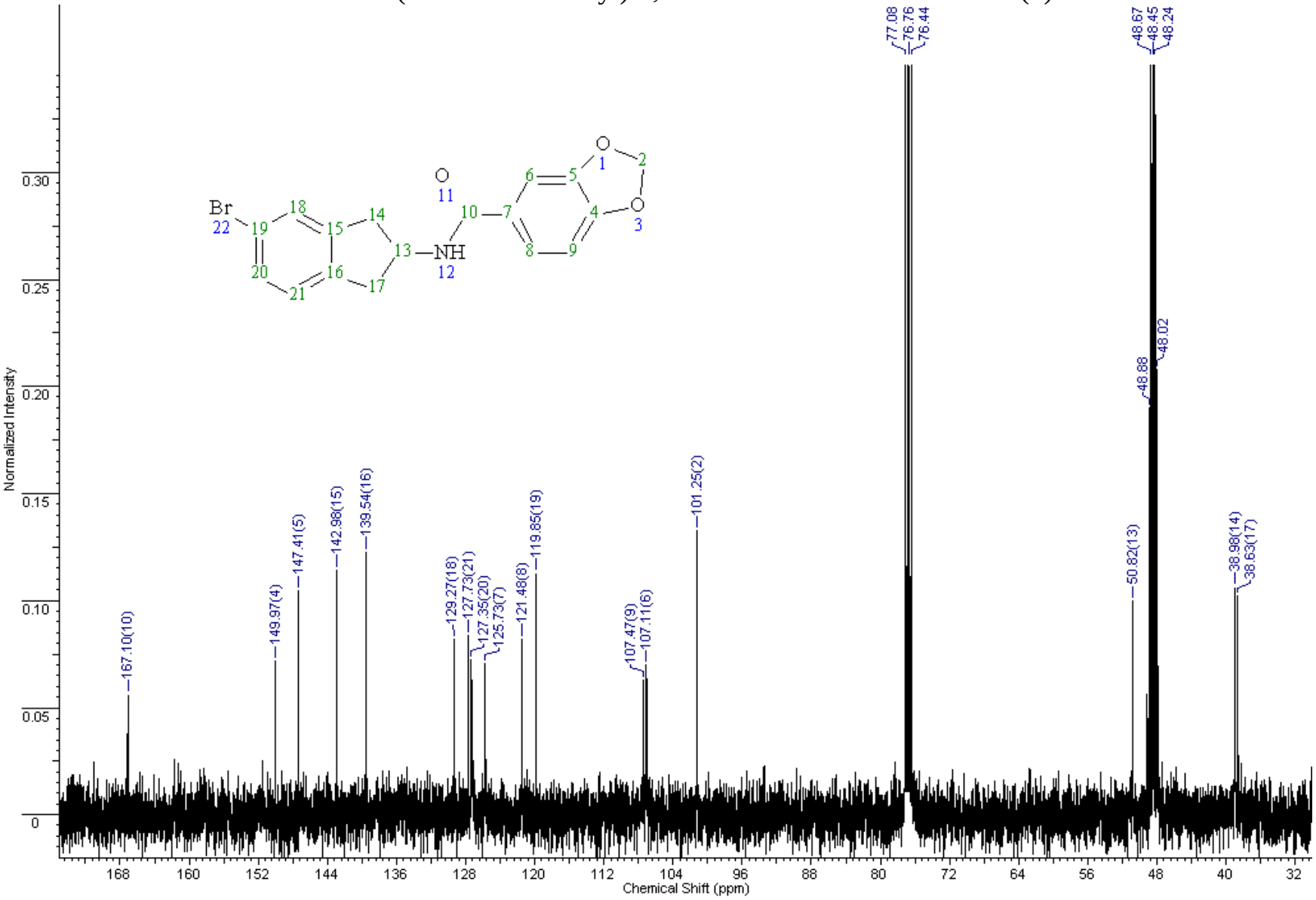
N-*{5-[(1,11-Dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradec-6-yl)methyl]-indan-2-yl}-1,3-benzodioxole-5-carboxamide* **1**. A 20% aqueous solution of KOH was added to a suspension of *N*-(5-(aminomethyl)indan-2-yl)-1,3-benzodioxole-5-carboxamide hydrochloride **7** (86 mg, 0.248 mmol) in distilled water (pH 10). The resulting mixture was extracted with CH_2Cl_2, the combined organic phases were dried over anhydrous Na_2SO_4, then filtered and the solvent was distilled off. The remainder free amine **7'** was dissolved in NMP (15 ml), then K_2CO_3 (319 mg, 2.31 mmol) and 3,7-bis(chloroacetyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one **8** (88 mg, 0.273 mmol) were added. The reaction mixture was stirred under heating at 75 °C for 15 hours. Then the reaction mixture was cooled to room temperature and filtered, the solvent was distilled off with an oil pump. The solid residue was treated with Et_2O and filtered off. The resulting product was purified by column chromatography (eluents $CHCl_3$, $CHCl_3$/EtOH (100:1) and $CHCl_3$/EtOH (50:1)). This yielded 80 mg (0.143 mmol, 58%) of pure *N*-(5-((1,11-dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradec-6-yl)methyl)indan-2-yl)-1,3-benzodioxole-5-carboxamide **1** as a white crystalline product. M.p. 173–174°C. ^{1}H NMR ($CDCl_3$), δ: 1.01 (s, 3H), 1.09 (s, 3H), 2.74 (d, 2H, ^{2}J 13.4 Hz), 2.90—2.95 (m, 2H), 3.02 (d, 2H, ^{2}J 13.4 Hz), 3.21—3.27 (m, 2H), 3.39—3.46 (m, 2H), 3.59 (s, 2H), 3.78 (m, 2H), 4.93 (m, 4H), 6.02 (s, 2H), 6.17 (d, 1H, ^{3}J 7.2 Hz), 6.81 (d, 1H, ^{3}J 8.3 Hz), 7.15—7.29 (m, 5H). ^{13}C NMR ($CDCl_3$), δ: 15.41, 15.80, 39.36, 39.53, 44.94, 45.39, 50.85, 53.91, 55.04, 59.91, 60.00, 62.01, 101.29, 107.19, 107.54, 121.16, 124.69, 126.05, 127.91, 128.14, 134.05, 141.01, 141.42, 147.52, 149.93, 166.24, 167.79, 210.52. HRMS (ESI), m/z 559.2550 (calc. for $C_{31}H_{34}N_4O_6$ $[M+H]^+$, m/z: 559.2551).

2. NMR Spectra

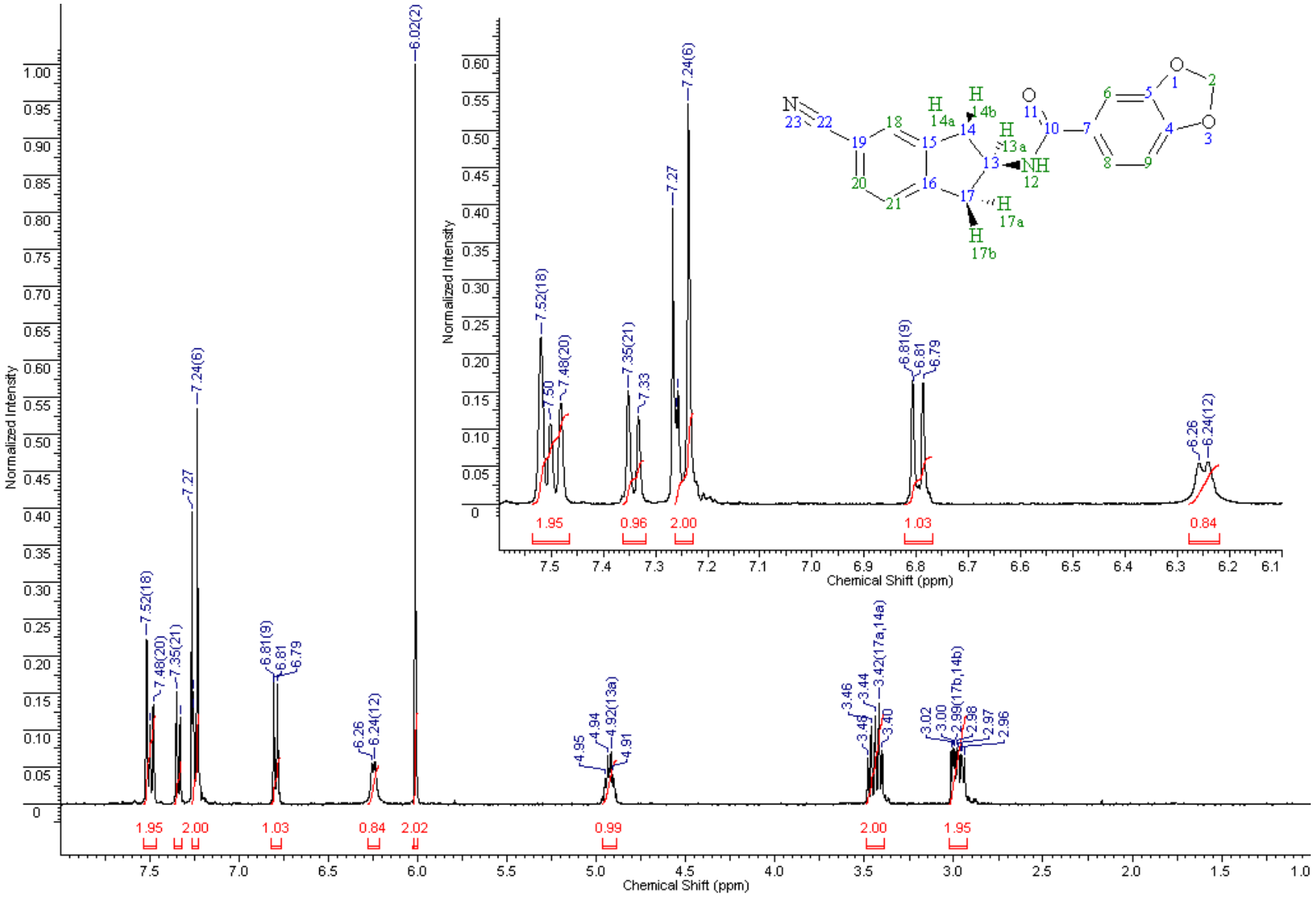
^{1}H NMR *N*-(5-bromoindan-2-yl)-1,3-benzodioxole-5-carboxamide (**5**).



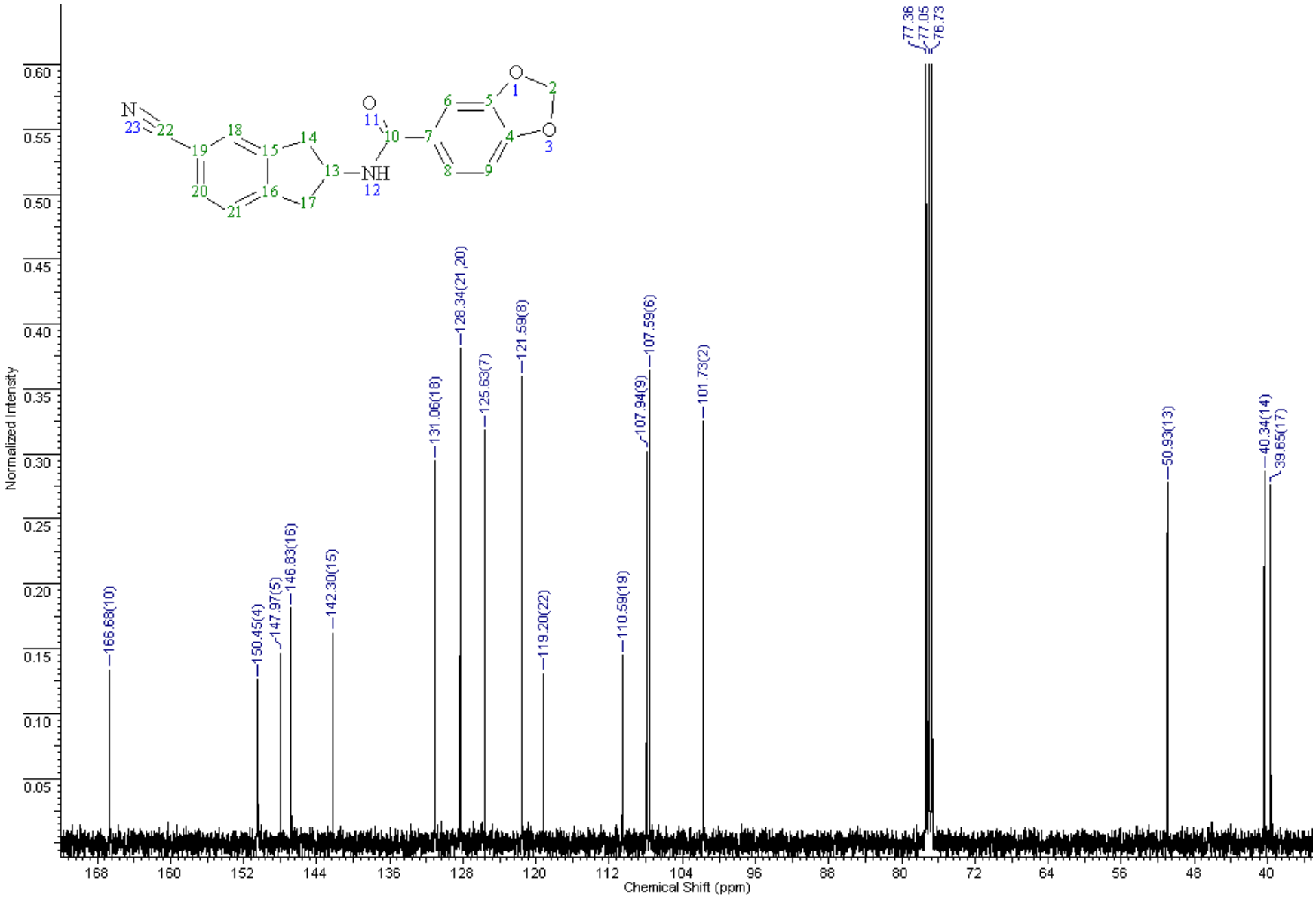
^{13}C NMR *N*-(5-bromoindan-2-yl)-1,3-benzodioxole-5-carboxamide (**5**).



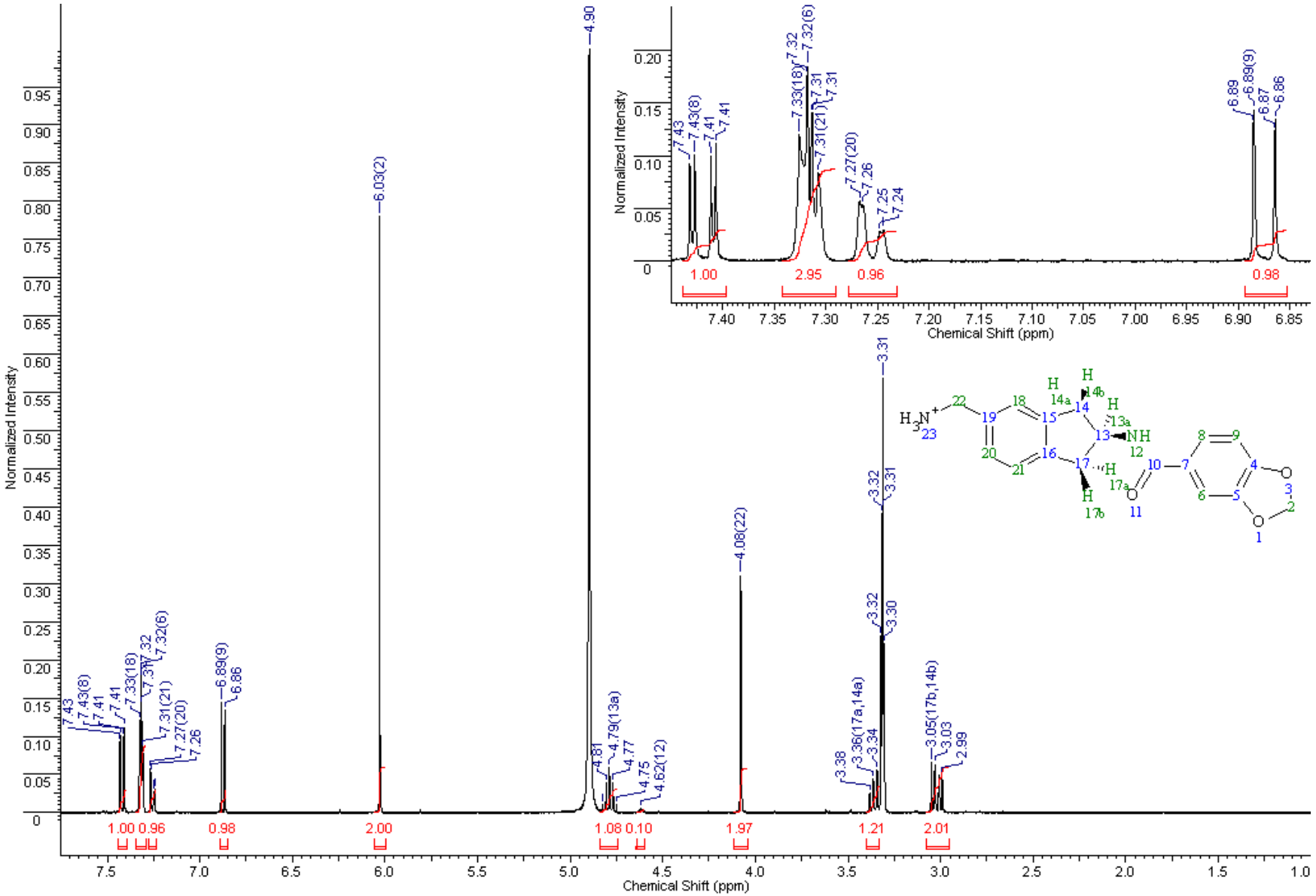
^{1}H NMR *N*-(5-cyanoindan-2-yl)-1,3-benzodioxole-5-carboxamide (**6**).



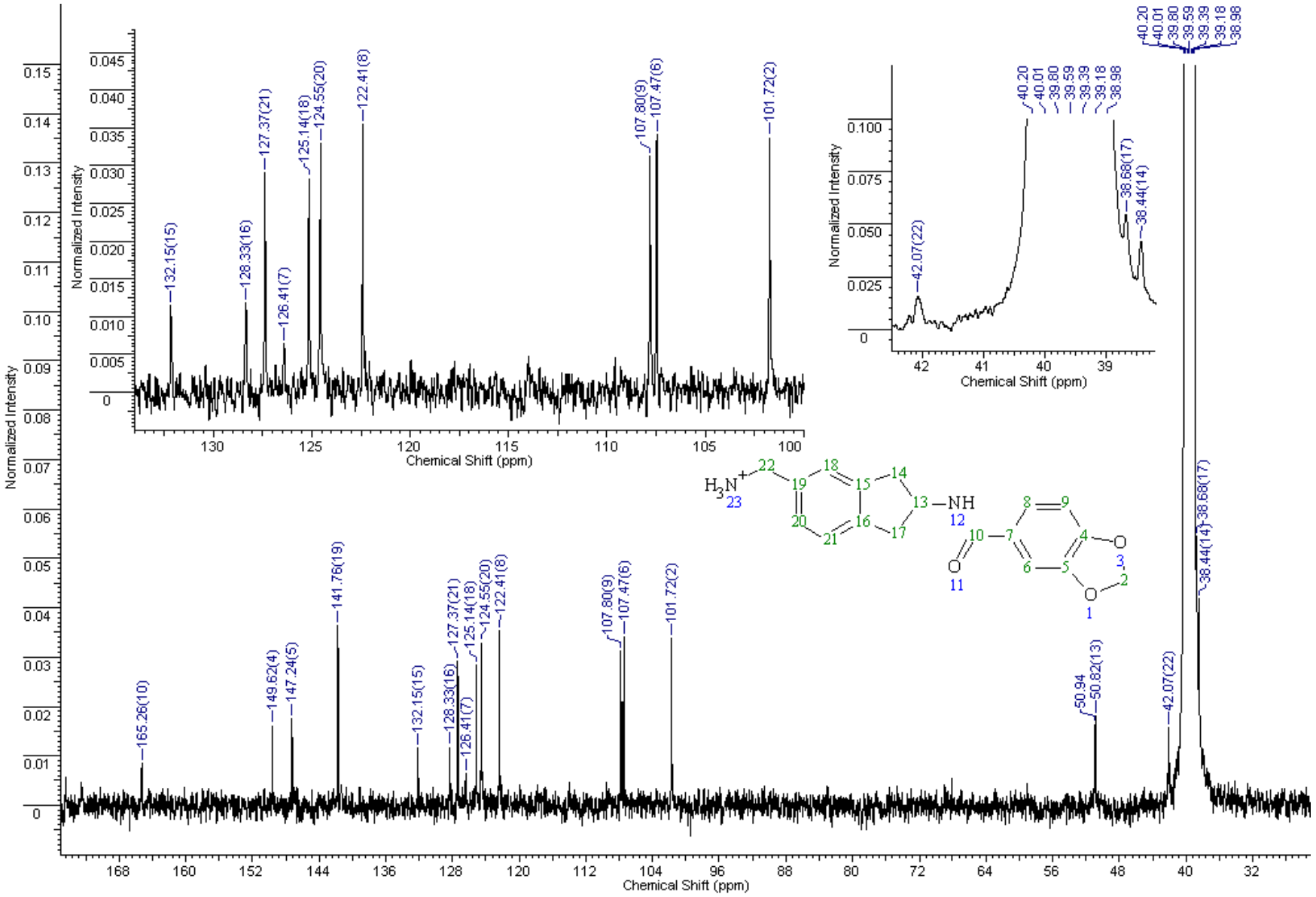
^{13}C NMR *N*-(5-cyanoindan-2-yl)-1,3-benzodioxole-5-carboxamide (**6**).



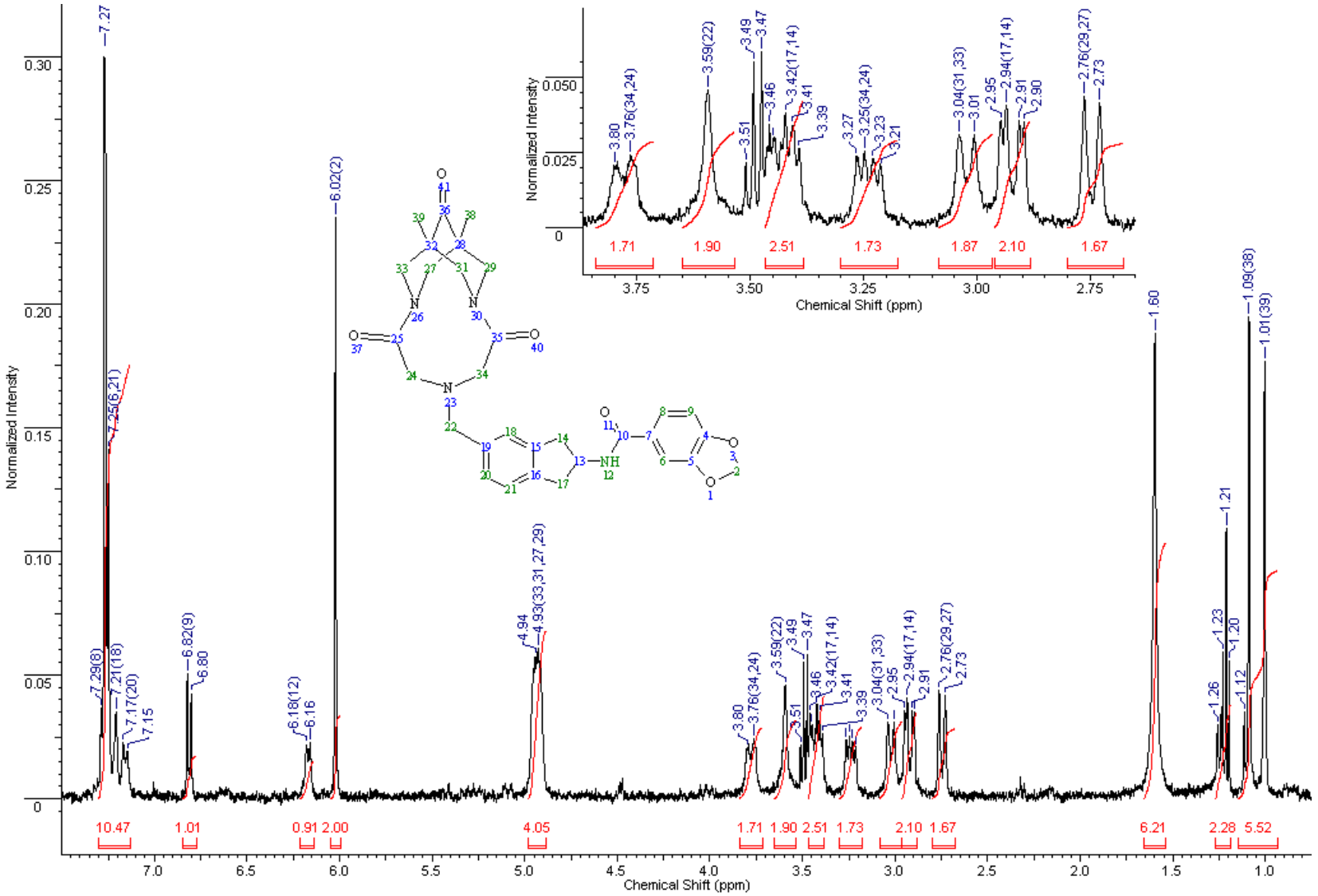
^{1}H NMR *N*-[5-(aminomethyl)indan-2-yl]-1,3-benzodioxole-5-carboxamide hydrochloride (**7**).



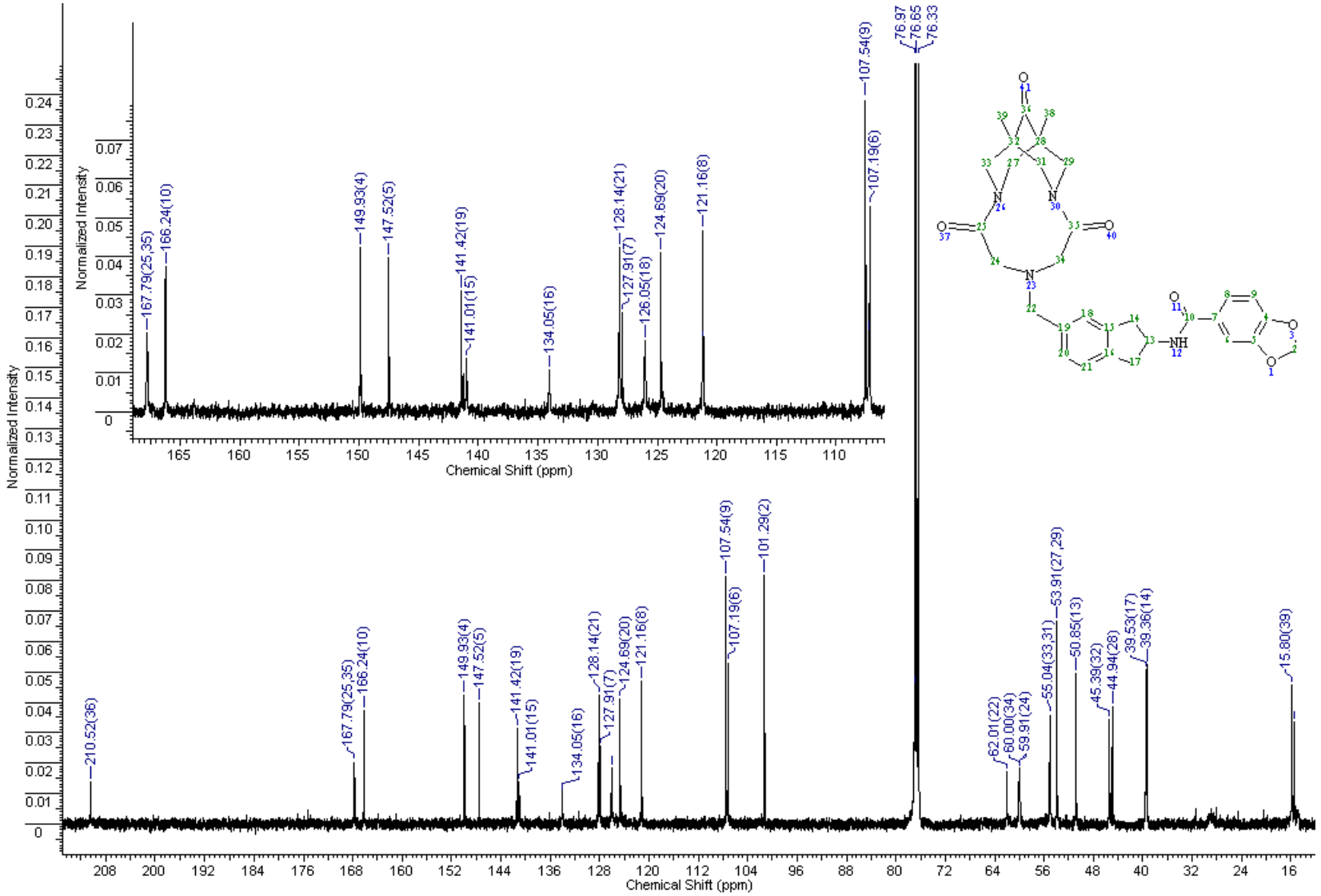
^{13}C NMR *N*-[5-(aminomethyl)indan-2-yl]-1,3-benzodioxole-5-carboxamide hydrochloride (**7**).



^{1}H NMR *N*-{5-[(1,11-dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradec-6-yl)methyl]indan-2-yl}-1,3-benzodioxole-5-carboxamide (**1**).



^{13}C NMR *N*-{5-[(1,11-dimethyl-4,8,12-trioxo-3,6,9-triazatricyclo[7.3.1.1^{3,11}]tetradec-6-yl)methyl]indan-2-yl}-1,3-benzodioxole-5-carboxamide (**1**).



3. Molecular modelling

The structure of the dimeric ligand-binding domain of the rat GluA2 AMPA receptor was obtained from the Protein Data Bank (PDB: 4FAT).[S5] Upon removal of ions and small molecules (except the two bound glutamate agonist molecules), the protein was allowed to relax during molecular dynamics simulation for 100 ns (see below for the simulation protocol). The most frequently occurring structure was identified by clustering of the frames in the stable part of the trajectory (40–100 ns). The ligand structures were converted to 3D and preoptimized in the MMFF94 force field using Avogadro 1.2.0 software,[S6] and then the ligand and protein structures were prepared for molecular docking using AutoDock Tools 1.5.6 software.[S7] Molecular docking into the positive allosteric modulator binding site was performed with AutoDock Vina 1.1.2 software[S8] (grid box size 22 Å × 29 Å × 40 Å, exhaustiveness = 16). The pose with the best scoring function value and ligand position was selected and the complex model was built using the UCSF Chimera 1.15 software.[S9]

The molecular dynamics simulations were performed using the CHARMM36 / CGenFF 4.6 force field[S10,S11] in the GROMACS 2021.2 software.[12] The initial models of the systems were built using the Ligand Reader & Modeler and Solution Builder modules of the CHARMM-GUI web service.[S13,S14] The protein molecule was inserted into a rectangular periodic boundary box of water in the TIP3P model; the distance from the protein to the box border was no less than 10 Å. Individual randomly selected water molecules were replaced with potassium and chlorine ions to ensure electrical neutrality of the system and the total concentration of KCl about 0.15 M. For each system, the molecular mechanics minimization (up to 5000 steps) was performed on the CPU, followed by equilibration for 125 ps with integration timestep of 1 fs at the temperature of 300 K and constant volume using the v-rescale thermostat on the NVIDIA GeForce RTX 3080 GPU. The production simulation was performed on the GPU with integration timestep of 2 fs at the temperature of 300 K and the constant pressure of 1 bar using the v-rescale thermostat and the Parrinello–Rahman barostat. The hydrogen atom movements were constrained using the LINCS algorithm. For the analysis and visualization of the results, the cpptraj software[S15] in the AmberTools 22 package[S16] and UCSF Chimera were used.

The plots of the root mean square deviations (RMSD) for the protein, glutamate, and ligand heavy atoms (Figure S1) as well as the visual inspection of the trajectories confirm that the system stability is retained over the entire course of the production simulation (100 ns). The position of the ligand **1** in the PAM binding site at the interface between the dimeric ligand-binding domains is slightly adjusted compared to the docking pose, and its binding mode remains stable over the entire course of the simulation. Similar to other larger-sized modulators,[S17] the **1** molecule occupies the central and side subpockets of the symmetrical PAM binding site (main text Figure 2, parts *a*, *b*). The binding is primarily stabilized by hydrophobic interactions and steric fit (parts *b*, *c*).

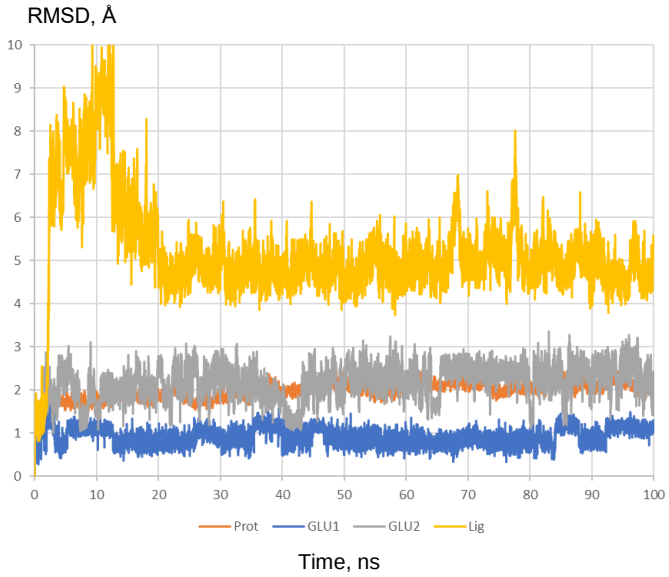


Figure S1 RMSD of the protein, glutamate, and ligand **1** non-hydrogen atoms during molecular dynamics simulation of its complexe with the PAM binding site of the dimeric ligand-binding domain of the GluA2 AMPA receptor.

The binding free energies were estimated over the stable portion of the trajectories (last 20 ns, 101 frames at 200 ps interval) using the MM/GBSA approach implemented in the gmx_MMPBSA 1.4.3 software.[S18,S19] The internal dielectric constant $\varepsilon = 4$ and the Interaction Entropy model for the conformation entropy contribution were used. The resulting energy values are listed in Table S1.

Overall, these results indicate that the compound **1** can indeed act as a positive AMPA receptor modulator which binds in the validated PAM binding site.

Table S1 Binding free energy for compound **1** calculated using MM/GBSA approach.

Energy terms, kcal mol^{-1}	Compound **1**
ΔE_{int}	0±0
ΔE_{ele}	0.06±0.3
ΔE_{vdw}	–45.6±0.3
$\Delta E_{MM} = \Delta E_{int} + \Delta E_{ele} + \Delta E_{vdw}$	–45.5±0.4
ΔG_{GB}	6.5±0.3
ΔG_{SA}	–5.76±0.03
$\Delta G_{sol} = \Delta G_{GB} + \Delta G_{SA}$	0.8±0.3
$\Delta G_{MMGBSA} = \Delta E_{MM} + \Delta G_{sol}$	–44.8±0.3
$- T\Delta S$	6.2±0.2
$\Delta G_b = \Delta G_{MMGBSA} - T\Delta S$	–38.6±0.3

Note: Values are listed as Mean ± Standard Error of Mean.

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