Supporting Information:

Design and Synthesis of 2,3-*trans*-Proline Analogs as Ligands for the Ionotropic Glutamate Receptors and the Excitatory Amino Acid Transporters

Christian B. M. Poulie[a], Anna Alcaide[a], Mikkel Krell-Jørgensen[a], Younes Larsen[a], Eloi Astier[a], Walden E. Bjørn-Yoshimoto[a], Feng Yi[b], Jed T. Syrenne[b], Morten Storgaard[a], Birgitte Nielsen[a], Karla A. Frydenvang[a], Anders A. Jensen[a], Kasper B. Hansen[b], Darryl S. Pickering[a] and Lennart Bunch*[,a]

[a] *Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark*

[b] *Department of Biomedical and Pharmaceutical Sciences, Center for Structural and Functional Neuroscience, and Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT 59812, USA.*

Content:

X-ray crystallographic analysis of compound 9c-s1

Crystal data: single crystals suitable for X-ray diffraction studies were grown from a solution in EtOAc/heptane, $C_{14}H_{23}NO_6$, M_r 301.34, orthorhombic, space group $P2_12_12_1$ (No 19), a = 6.2820(8), b = 11.901(3), c = 21.253(2) Å, V = 1588.9(4) Å^3, Z = 4, D_c = 1.260 Mg/m^3, F(000) = 648, μ(Mo$K\alpha$) = 0.098 mm^{-1}, crystal size: 0.3 x 0.16 x 0.15 mm.

Data collection and reduction: a single crystal was mounted and immersed in a stream of nitrogen gas [T = 123(1)K]. Data were collected, using graphite monochromated Mo$K\alpha$ radiation source (λ = 0.71073 Å) on a KappaCCD diffractometer. Data collection and cell refinement were performed using COLLECT (1) and DIRAX (2). Data reduction was performed using EvalCCD (3). Correction for absorption was performed using Gaussian integration (4,5) (Table S1).

Structure solution and refinement: positions of all non-hydrogen atoms were found by direct methods (SHELXS97) (6). Full-matrix least squares refinements (SHELXL) (7), were performed on F^2, minimizing $\Sigma w(F_o^2\text{-}kF_c^2)^2$, with anisotropic displacement parameters of the non-hydrogen atoms. The position of the hydrogen atoms were located in subsequent difference electron density maps and refined with fixed isotropic displacement parameters (U_{iso}= 1.2U_{eq} for CH, U_{iso} = 1.5U_{eq} for OH), except for the hydrogen atoms bonded to the methylene and methyl. These hydrogen atoms have been included in calculated position with fixed isotropic displacement parameters (U_{iso} = 1.2U_{eq} for CH_2, U_{iso} = 1.5U_{eq} for CH_3). Refinement (210 parameters, 3636 unique reflections) converged at R_F = 0.042, wR_F^2 = 0.089 [3222 reflections with $F_o > 4\sigma(F_o)$; $w^{-1} = (\sigma^2(F_o^2)+(0.0325P)^2 + 0.6062P)$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.090]. Non-centrosymmetric space group is assigned, but the absolute configuration cannot be determined (Flack = -1.2(13) (8). However the chirality for two chiral centers is known, and the third center is assigned relative to those (Fig. S1). The residual electron density varied between -0.18 and 0.21 e Å^{-3}. Complex scattering factors for neutral atoms were taken from International Tables for Crystallography as incorporated in SHELXL (7,9).

Fractional atomic coordinates, list of anisotropic displacement parameters and a complete list of geometrical data for compound **9c-s1** have been deposited in Cambridge Crystallographic Data Centre (CCDC 1893837). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

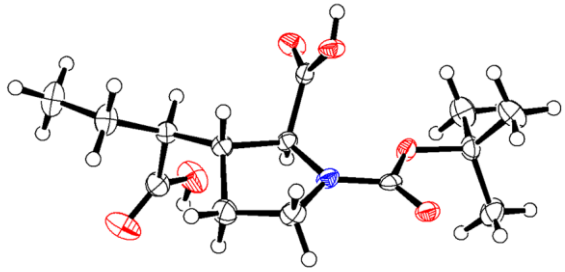

Fig.S1

Perspective drawing (ORTEP-3, (10)) of compound **9c-s1.** Displacement ellipsoids of the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size. Oxygen atoms are red and nitrogen atom blue.

X-ray crystallographic analysis of compound 9d-s2

Crystal data: single crystals suitable for X-ray diffraction studies were grown from a solution in EtOAc/heptane, $C_{18}H_{23}NO_6$, M_r 349.37, monoclinic, space group $P2_1$ (No 4), a = 12.0859(5), b = 5.7193(2), c = 13.4234(7) Å, β = 107.117(2)°, V = 886.76(7) Å^3, Z = 2, D_c = 1.308 Mg/m^3, F(000) = 372, μ(Mo$K\alpha$) = 0.098 mm^{-1}, crystal size: 0.22 x 0.11 x 0.09 mm.

Data collection and reduction: A single crystal was mounted and immersed in a stream of nitrogen gas [T = 123(1) K]. Data were collected, using graphite-monochromated Mo$K\alpha$ radiation (λ = 0.71073 Å) on a Bruker D8 Venture diffractometer. Data collection and cell refinement were performed using the Bruker Apex2 Suite software.(11) Data reduction using SAINT (12) and multi-scan correction for absorption using SADABS-2012-1 (13) were performed within the Apex2 Suite. The crystal data, data collection and the refinement data are given in Table S1.

Structure solution and refinement: positions of all non-hydrogen atoms were found by direct methods (SHELXS97) (6). Full-matrix least squares refinements (SHELXL) (7), were performed on F^2, minimizing $\Sigma w(F_o^2\text{-}kF_c^2)^2$, with anisotropic displacement parameters of the non-hydrogen atoms. The position of the hydrogen atoms were located in subsequent difference electron density maps and refined with fixed isotropic displacement parameters (U_{iso}= 1.2U_{eq} for CH, U_{iso} = 1.5U_{eq} for OH), except for the hydrogen atoms bonded to the aromatic, methylene and methyl moieties. These hydrogen atoms have been included in calculated position with fixed isotropic displacement parameters (U_{iso} = 1.2U_{eq} for CH and CH_2, U_{iso} = 1.5U_{eq} for CH_3). Refinement (245 parameters, 3017 unique reflections) converged at R_F = 0.040, wR_F^2 = 0.098 [2672 reflections with $F_o > 4\sigma(F_o)$; $w^{-1} = (\sigma^2(F_o^2)+(0.0583P)^2 + 0.1055P)$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.049]. Non-centrosymmetric space group is assigned, but the absolute configuration cannot be determined (Flack = 0.2(15) (8). However the chiralities for two chiral centers are known, and the third center is assigned relative to those (Fig. S2). The residual electron density varied between -0.20 and 0.36 e $Å^{-3}$. Complex scattering factors for neutral atoms were taken from International Tables for Crystallography as incorporated in SHELXL (7,9).

Fractional atomic coordinates, list of anisotropic displacement parameters and a complete list of geometrical data for compound **9d-s2** have been deposited in Cambridge Crystallographic Data Centre (CCDC 1893836). Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

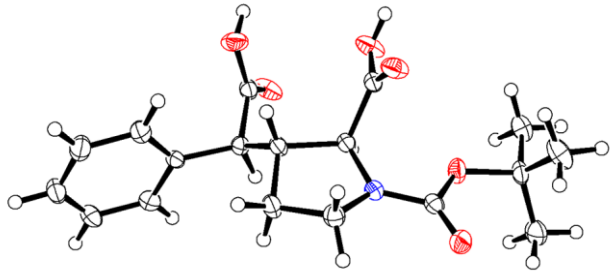

Fig.S2

Perspective drawing (ORTEP-3, (10)) of compound **9d-s2.** Displacement ellipsoids of the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size. Oxygen atoms are red and nitrogen atom blue.

References

1. COLLECT, B. V. Nonius Delft, The Netherlands. 1999

2. Duisenberg, A. J. M., 1992. Indexing in single-crystal diffractometry with an obstinate list of reflections. J. Appl. Crystallogr. 25, 92-96.

3. Duisenberg, A. J. M., 1998. EvalCCD. PhD thesis, University of Utrecht, The Netherlands.

4. Coppens, P. Evaluation of absorption and extinction in single-crystal structure analysis. In *Crystallogr. Comput., Proc. Int. Summer Sch.*; Hall, S. R. Ed.; Munksgaard: Copenhagen, 1970; pp 255-270. (included in the program package MaXus)

5. Nonius 2003, maXus 1.2.1. Nonius BV, Delft, The Netherlands (procedure NUMABS).

6. Sheldrick, G. M. A short history of SHELX. Acta Crystallogr., Sect. A: Found. Crystallogr. 2008, 64, 112-122.

7. Sheldrick, GM. (2015) "Crystal structure refinement with SHELXL", Acta Crystallogr. Sect. C: Struct. Chem. 2015, C71, 3-8.

8. Flack, H. D. On enantiomorph-polarity estimation. Acta Crystallogr., Sect. A: Found. Crystallogr. 1983, A39, 876-81.

9. International Tables for Crystallography; Wilson, A. J. C., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995; Vol. C, Tables 4.2.6.8 and 6.1.1.4.

10. Farrugia, L. J. ORTEP-3 for windows - a version of ORTEP-III with a graphical user interface (GUI). J. Appl. Crystallogr. 1997, 30, 565.

11. Bruker 2013. Apex2 Suite. Bruker AXS Madison, W., USA.

12. Bruker 2013. SAINT Version 8.30C. Data reduction and correction program. Bruker AXS Inc. Madison, W., USA.

13. Bruker 2012. SADABS-2012/1. Bruker/Siemens Area Detector Absorption Correction program. Bruker AXS Inc. Madison, W., USA.

Acknowledgments

The technical assistance of Niels Vissing Holst and Anders Kadziola, Department of Chemistry, University of Copenhagen, with collecting X-ray data and data reduction is gratefully acknowledged.

Table S1. Crystal data, data collection and refinement data for compound 9c-s1 and compound 9d-s2.

	9c-s1	**9d-s2**
Formula	$C_{14}H_{23}NO_6$	$C_{18}H_{23}NO_6$
Mw (g/mol)	301.34	349.37
Temperature, K	123(1)	123(1)
Crystal class	ortorhombic	monoclinic
Space group	$P2_12_12_1$	$P2_1$
Cell parameters	a = 6.2829(8) Å b = 11.901(3) Å c = 21.253(2) Å	a = 12.0859(5) Å b = 5.7193(2) Å c = 13.4234(7) Å β = 107.117(2)°
V (Å^3), Z	1588.9(4) / 4	886.76(7) / 2
F(000)	648	372
d_{calc} (g/cm^3)	1.260	1.308
λ	0.71073 Å (MoKα)	0.71073 Å (MoKα)
θ_{max} (°)	5.893 < θ < 27.562	2.698 < θ < 24.676
Reflections total/unique	37335/3636	9881/3017
R_{merge}	0.069	0.0743
No. parameters/restraints	210/0	245/1
Reflections (I > 2 σ(I))	3222	2672
R1/*wR2* (I > 2 σ(I))	0.0422/0.0888	0.0403/0.0981
R1/*wR2* (all reflections)	0.0516/0.0930	0.0489/0.1044
Goodness-of-fit on F^2	1.090	1.049
ρ_{max} / ρ_{min} (eÅ^{-3})	0.21 / -0.18	0.36 / -0.20
Flack parameter	-1.2(13)	0.2(15)