Concise Asymmetric Synthesis and Pharmacological Characterization of All Stereoisomers of Glutamate Transporter Inhibitor TFB-TBOA and Synthesis of EAAT Photoaffinity Probes

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Ⓢ *Supporting Information*

ABSTRACT: Glutamate is the major excitatory neuro-transmitter in the mammalian brain. Its rapid clearance after the release into the synaptic cleft is vital in order to avoid toxic effects and is ensured by several transmembrane transport proteins, so-called excitatory amino acid transporters (EAATs). Impairment of glutamate removal has been linked to several neurodegenerative diseases and EAATs have therefore received increased attention as therapeutic targets. O-Benzylated L-*threo*-β-hydroxyaspartate derivatives have been developed previously as highly potent inhibitors of EAATs with TFB-TBOA ((2S,3S)-2-amino-3-((3-(4-(trifluoromethyl)-benzamido)benzyl)oxy)succinic acid) standing out as low-nanomolar inhibitor. We report the stereoselective synthesis of all four stereoisomers of TFB-TBOA in less than a fifth of synthetic steps than the published route. For the first time, the inhibitory activity and isoform selectivity of these TFB-TBOA enantio- and diastereomers were assessed on human glutamate transporters EAAT1−3. Furthermore, we synthesized potent photoaffinity probes based on TFB-TBOA using our novel synthetic strategy.

KEYWORDS: Glutamate transporters, asymmetric synthesis, inhibitors, aspartate derivatives, isoform selectivity, photoaffinity probes
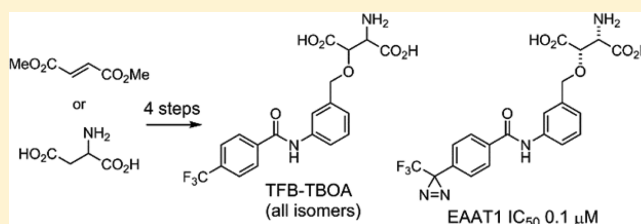
The involvement of glutamate in electrophysiological processes in the brain was first proposed and later confirmed in the 1950s.[1] It has been established since that the glutamatergic system regulates most of the excitatory activities in the central nervous system and it is estimated that 80−90% of the neurons use glutamate as neurotransmitter. A crucial step is the rapid termination of glutamate excitation since a high concentration of glutamate in the synaptic cleft, as upon release from presynaptic vesicles, is leading to excitotoxicity over time, which in turn can result in neuronal damage.[2]

Rapid removal of extracellular glutamate is achieved by five selective transmembrane transport proteins belonging to the solute carrier (SLC) 1 family: EAAT1 (human gene *SLC1A3*, termed GLAST in rodents), EAAT2 (*SLC1A2*, GLT-1), EAAT3 (*SLC1A1*, EAAC1), EAAT4 (*SLC1A6*), and EAAT5 (*SLC1A7*). The first three are generally expressed in the brain and play a fundamental role in the termination of glutamate-mediated excitation at synapses. The latter two are very tissue specific and only found in Purkinje cells in the cerebellum (EAAT4) and in retina (EAAT5), respectively. EAAT3 is mainly located on the surface of postsynaptic neurons, whereas EAAT1 and EAAT2 are expressed in astrocytes. EAAT2 alone is responsible for the transport of >90% of extracellular glutamate into astrocytes. Not surprisingly, impairment of the glial glutamate transporters in particular, is leading to excitotoxic effects and death of neurons.

In fact, several studies have linked the reduced function or expression of glutamate transporters to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer, Parkinson, and Huntington disease.[2,3] Thus, enhancing glutamate transport is a viable therapeutic strategy for neuroprotection, and corresponding studies both in vitro and in vivo in a mouse model of ALS, for instance, have provided some encouraging results.[4] Addressing glutamate transporters with modulators does not only pose a challenge in terms of isoform selectivity and dynamic expression levels; it has very recently been shown for GLT-1 at least, that they are highly mobile at the surface of astrocytes, which in turn shapes synaptic transmission.[5,6]

Several research groups have investigated the activity of small molecules, in particular L-glutamate (**1**) and L-aspartate (**2**) derivatives (Chart 1), to inhibit specific glutamate transporter isoforms.[7]
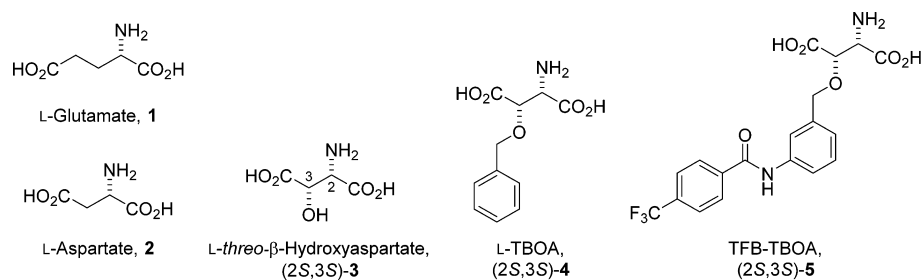
Early on, *threo*-L-β-hydroxyaspartate ((2S,3S)-**3**) was discovered to inhibit glutamate transport in synaptosomes and later identified as EAAC1 blocker.[8] Shimamoto and co-workers

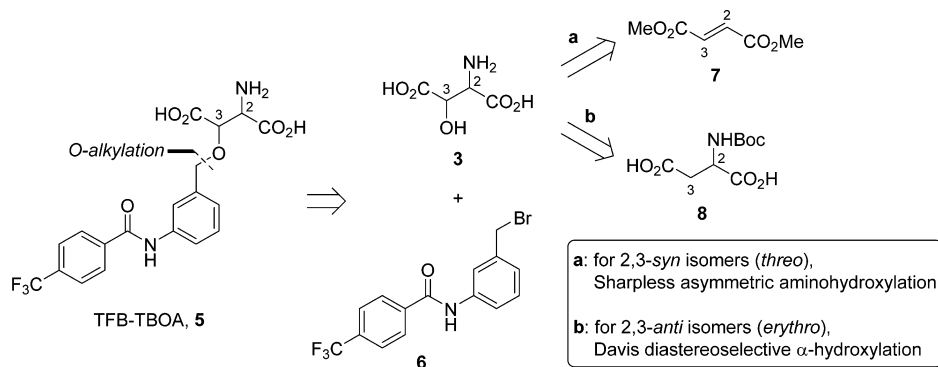**Chart 1. EAAT Transporter Substrates L-Glu and L-Asp and Inhibitors 3−5 Derived Thereof**

Scheme 1. Retrosynthetic Analysis and Synthetic Strategy to Access All Four Isomers of 5

a: for 2,3-*syn* isomers (*threo*), Sharpless asymmetric aminohydroxylation

b: for 2,3-*anti* isomers (*erythro*), Davis diastereoselective α-hydroxylation

developed a series of *threo*-L-β-hydroxyaspartate derivatives that are the most potent inhibitors of EAAT1−3 to date. From their first two studies, L-TBOA ((2S,3S)-**4**, L-*threo*-β-benzyloxyaspartate) emerged as the most active compound (IC$_{50}$ values in the low micromolar range).[9−11] L-TBOA was used in numerous neurobiological studies and, among other things, cocrystallized with the homologous prokaryotic aspartate transporter GltPh from *Pyroccocus hirokoshii* (PDB ID: 2NWW).[12] Notably, extending the structure at the phenyl ring, as exemplified by the best compound TFB-TBOA ((2S,3S)-**5**), yielded EAAT2 and EAAT3 inhibitors with IC$_{50}$ values in the low nanomolar range.[13]

Using the information from GltPh crystal structures,[12,14] other groups have used a structure-based screening approach to identify conformationally constrained analogues of aspartate and glutamate in a virtual compound library.[15] This led to the discovery of a norbornane-type aspartate derivative which exhibited an IC$_{50}$ of 1.4 μM for EAAT2 and showed no inhibition of EAAT3. Moving away from amino acid scaffolds, Bunch and co-workers synthesized a library of substituted 5-oxo-5,6,7,8-tetrahydro-4H-chromenes and identified congeners that were potent inhibitors of EAAT1 (IC$_{50}$ 0.43 μM for best compound) but displayed no noticeable inhibitory activity at EAAT2 and EAAT3.[16−18]

The published asymmetric syntheses of L-TBOA ((2S,3S)-**4**) and TFB-TBOA ((2S,3S)-**5**) are rather elaborate (10 and 21 synthetic steps, respectively).[11,19] Our goal was to establish a general and concise synthetic procedure to prepare derivatives of TFB-TBOA that we envision to develop into molecular probes for studying glutamate transporters in cells. Here, we report the synthesis of all four stereoisomers of TFB-TBOA (**5**) in only four synthetic steps and their inhibitory activity at human isoforms EAAT1−3. Furthermore, we used our novel synthetic strategy to generate two potent photoaffinity probes based on the TFB-TBOA scaffold.

■ RESULTS AND DISCUSSION

Chemistry. In our retrosynthetic analysis, the benzyl ether in **5** was disconnected which led to bromide **6** and the key intermediate β-hydroxyaspartate **3** (Scheme 1). After some literature research, we envisaged to use asymmetric amino-hydroxlyation developed by Sharpless and co-workers[20−22] to install the two stereogenic centers in one reaction with high *syn*-selectivity to access the *threo* (2,3-*syn*) isomers (TBOA, Scheme 1, retrosynthetic path a). For the projected *erythro* (2,3-*anti*) isomers (EBOA, Scheme 1, retrosynthetic path b), we chose to utilize the Davis α-hydroxylation methodology[23] to diastereoselectively install the stereogenic center at C-3. Thus, the projected synthetic plan would allow us to access all four stereoisomers of **5** in a very short four-step sequence starting either from fumarate **7** or protected aspartate **8**.

First, dimethyl fumarate (**7**) was converted to the *tert*-butyl diester **9** in order to streamline deprotection conditions at the end of the synthesis (Scheme 2). Following a recently published modification[24] of the Sharpless aminohydroxylation protocol yielded protected hydroxyaspartate **10** with complete diastereoselectivity (only *syn* isomers formed) in 65%. The major improvement of this protocol is the use of 4-chlorobenzoyloxycarbamate (**11**) instead of the commonly used N-chlorocarbamate[25] that has to be formed in situ from *tert*-butyl carbamate and freshly prepared *tert*-butyl hypochlorite under aqueous basic conditions. In the presence of chiral ligand (DHQD)$_2$PHAL the aminohydroxylation led to (2S,3S)-**10** as major enantiomer, whereas using the pseudoenantiomer (DHQ)$_2$PHAL as ligand gave mainly (2R,3R)-**10**. In both cases, the enantiomeric ratio (er) was determined to be 7:3 by chiral HPLC. It is important to run the aminohydroxylation reaction at −15 °C in order to suppress formation of 1,2-diol side products resulting from asymmetric dihydroxylation that is also operational under the reaction conditions. The free hydroxyl group of **10** was O-alkylated with bromide **6**[26] (for synthesis details, see the

Scheme 2. Synthesis of 2,3-syn Isomers (threo) of 5[a]
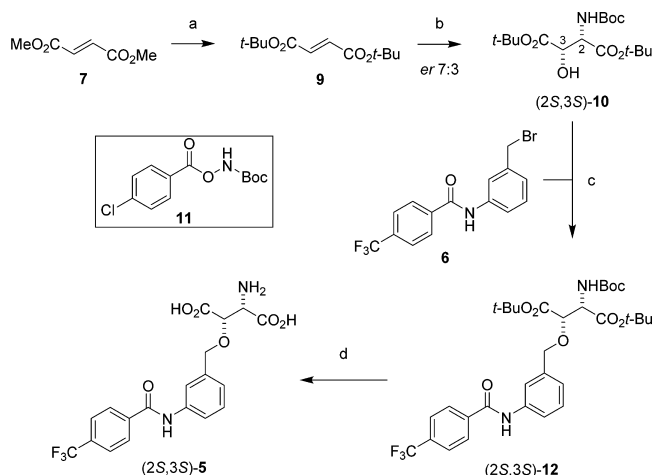
[a]Reagents and conditions: (a) t-BuOH, n-BuLi, THF, 0°C; then 7, 0°C to rt, 71%. (b) for (2S,3S)-10 (shown): OsO4, (DHQD)2PHAL, 11, H2O/MeCN, −15°C; then 9, H2O/MeCN, −15° to rt, 65%, er 70:30. For (2R,3R)-10 (not shown): OsO4, (DHQ)2PHAL, 11, H2O/MeCN, −15°C; then 9, H2O/MeCN, −15° to rt, 63%, er 69:31. (c) NaH, DMF, −15°C; then 6, −15°C to rt, 99% for (2S,3S)-12, 98% for (2R,3R)-12. (d) TFA, CHCl3, rt, 84% for (2S,3S)-5, quant. for (2R,3R)-5.

Supporting Information). Global deprotection using TFA in chloroform led to enantioenriched isomers (2S,3S)-5 or (2R,3R)-5. By following the same synthetic route as shown in Scheme 2 but omitting the chiral phthalazine ligand in the asymmetric aminohydroxylation step, we also synthesized the racemic mixture of the 2,3-syn (threo) isomers of 5 for biological assessment.

The synthesis of the two 2,3-anti (erythro) enantiomers started from enantiomerically pure N-Boc protected L- or D-aspartate that was first transformed into the corresponding di-tert-butyl ester 13 (Scheme 3) in order to simplify deprotection at the end.

Unexpectedly, this protection step failed or resulted in very low yields when employing commonly used conditions. A

Scheme 3. Synthesis of 2,3-anti Isomers (erythro) of 5[a]
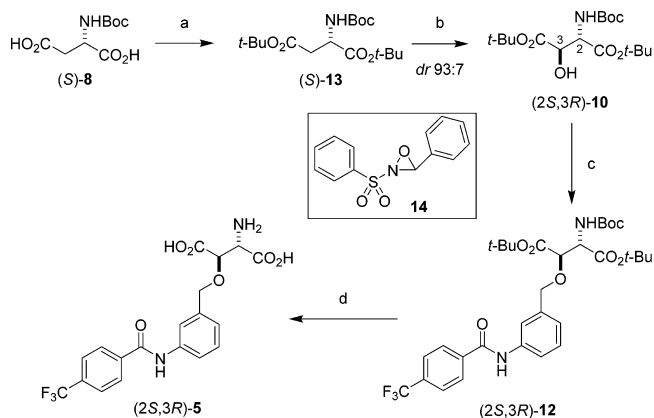
[a]Reagents and conditions: (a) CuCl, DIPC, t-BuOH; then 8, CH2Cl2, reflux, 79% for (S)-13, 97% for (R)-13. (b) NaHMDS, THF, −78°C; then 14, THF, −78°C, 22% and dr 93:7 for (2S,3R)-10, 26% and dr 92:8 for (2R,3S)-10. (c) NaH, 6, DMF, −15°C to 0°C, 19% for (2S,3R)-12, 21% for (2R,3S)-12. (d) TFA, CHCl3, rt, 82% for (2S,3R)-5, 96% for (2R,3S)-5.

recently published protocol[27] utilizing diisopropylcarbodiimide (DIPC), t-BuOH, and CuCl as reagents ultimately worked best for us. The α-hydroxylation reaction developed by Davis and co-workers is a powerful methodology to stereoselectively install hydroxyl groups next to carbonyl functions.[28] In our particular case, treatment of fully protected aspartate 13 with NaHMDS at low temperature generated the corresponding ester enolate which was then almost exclusively attacked by the oxaziridine 14 from the opposite face of the N-Boc group, yielding the 2,3-anti hydroxylated product with high diastereoselectivity (dr 92:8−93:7). The isolated yield of the α-hydroxylated products was low and a large amount of unreacted starting material was usually recovered. The O-alkylation of the 2,3-anti (erythro) alcohols 10 proved much more troublesome as we observed epimerization and elimination at reaction temperatures above 5 °C. Monitoring epimerization (by ¹H NMR) is advisable since the separation of diastereomers 2,3-anti-12 and 2,3-syn-12 is extremely difficult. Interestingly, once purified and isolated, the 2,3-anti isomers of 12 appear to be configurationally stable even at elevated temperature (60 °C for 24 h in DMSO-d_6). Global deprotection to yield the final compounds (2S,3R)-5 and (2R,3S)-5 was achieved by treatment with TFA in chloroform. Notably, the 2,3-anti isomers did not epimerize under the strong acidic conditions. For the biological assessment we also synthesized the racemic mixture of the 2,3-anti isomers of 5 by starting from racemic N-Boc aspartate and following the synthetic route depicted in Scheme 3.

The synthetic route depicted in Scheme 2 is amenable to rapidly access TFB-TBOA analogues. For instance, by alkylating alcohol (2S,3S)-10 with the appropriate building blocks we generated diazirine- and benzophenone-TFB-TBOA photo-affinity probes 15 and 16 (Chart 2), respectively (for details of

Chart 2. Structures of EAAT Photoaffinity Probes 15 and 16
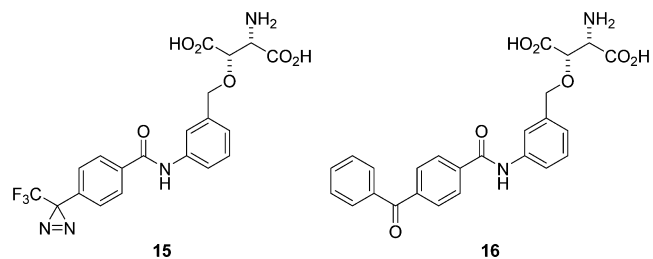
their synthesis, see the Supporting Information). Diazirine and benzophenone moieties appended to pharmacophores are commonly used for photo-cross-linking studies with proteins and can help to map the binding site of the pharmacophore.[29,30]

Biological Activity. With all four stereoisomers of 5 in hand, we next examined their ability to inhibit glutamate uptake into cells. To this end, HEK293 cell lines stably expressing either human EAAT1, 2, or 3 were generated and transport of [³H]-L-glutamate into these cells in the presence of 5 was determined using a similar assay set up as described previously (using 10 μM L-glutamate in the transport assay).[31] For all four stereoisomers of 5, we observed dose-dependent inhibition of [³H]-L-glutamate transport (Figure 1).

Overall, the 2,3-syn (threo, TFB-TBOA) isomers of 5 are more potent inhibitors of glutamate uptake facilitated by EAAT1, 2, or 3 than the 2,3-anti (erythro, TFB-EBOA) isomers by 1−2 orders of magnitude (Table 1). This is in line with what was observed for the stereoisomers of β-benzyloxyaspartate, where for instance L-TBOA ((2S,3S)-4) was a more active inhibitor than L-EBOA
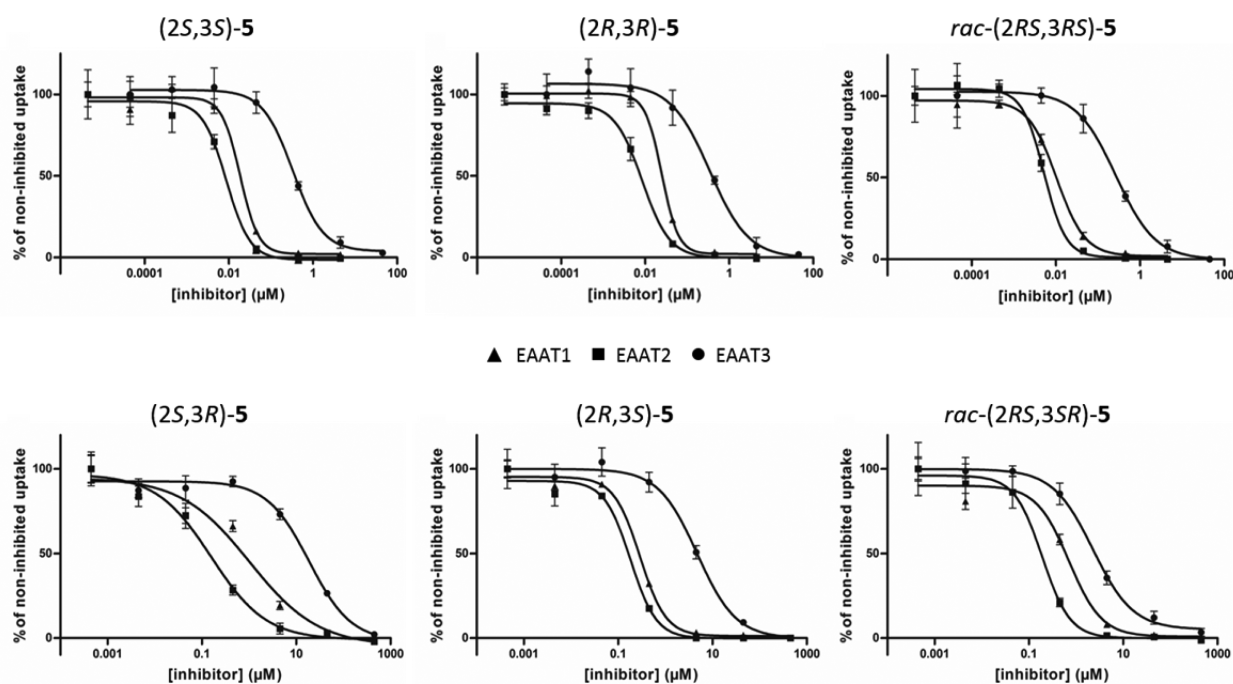
Figure 1. Dose-dependent inhibition of $[^3H]$-L-glutamate transport mediated by EAAT1 (▲), EAAT2 (■), or EAAT3 (●), by stereoisomers of **5**. Top panels: 2,3-*syn* isomers (*threo*, TFB-TBOA). Bottom panels: 2,3-*anti* isomers (*erythro*, TFB-EBOA). Dose−inhibition curves shown are representative experiments. L-Glutamate concentration used in the transport assay was 10 μM. All data are means of three measurements ± SD.

Table 1. Inhibiton of Human Glutamate Transporter-Mediated $[^3H]$-L-Glutamate Uptake by TFB-TBOA and TFB-EBOA Stereoisomers[a]

	compd	IC$_{50}$ (nM)		
		EAAT1	EAAT2	EAAT3
2,3-*syn*, *threo* TFB-TBOA	commercial (*S*,*S*)-TFB-TBOA	8.2 ± 3.6	9.3 ± 1.0	248 ± 76
	synthetic TFB-TBOA, (2*S*,3*S*)-**5**[b]	20.7 ± 3.9	9.9 ± 1.3	284 ± 34
	(2*R*,3*R*)-**5**[b]	19.2 ± 4.2	12.2 ± 4.1	412 ± 86
	rac-(2*RS*,3*RS*)-**5**	10.1 ± 1.5	6.4 ± 1.8	228 ± 37
2,3-*anti*, *erythro* TFB-EBOA	(2*S*,3*R*)-**5**	1051 ± 97	175 ± 65	13824 ± 5007
	(2*R*,3*S*)-**5**	335 ± 96	181 ± 68	2399 ± 1690
	rac-(2*RS*,3*SR*)-**5**	619 ± 73	179 ± 88	5208 ± 2830

[a]L-Glutamate concentration used in the transport assay was 10 μM. All data are means of at least three independent experiments performed in triplicate ± SD. [b]Enantioenriched, er 7:3.

((2*S*,3*R*)-**4**).[11] It is interesting to note, however, that the difference in inhibitory potency of the β-benzyloxyaspartate stereoisomers **4** is more or less pronounced depending on the glutamate transporter isoform studied; an observation that we have also made with our synthesized stereoisomers of **5**.

In terms of isoform selectivity, EAAT2 was inhibited most strongly by all four stereoisomers of **5**, followed by EAAT1 and then EAAT3, for the latter of which we obtained IC$_{50}$ values in the high nanomolar to low micromolar range.

The IC$_{50}$ values for our synthetic TFB-TBOA ((2*S*,3*S*)-**5**), even though only enantioenriched, were almost identical for EAAT2 and EAAT3 and very similar for EAAT1 compared to the values we obtained with commercially available (*S*,*S*)-TFB-TBOA. Furthermore, the IC$_{50}$ values obtained with our assay are very comparable to literature values for (*S*,*S*)-TFB-TBOA, where $[^{14}C]$-L-glutamate uptake into COS-1 cells transiently expressing EAAT1, 2, or 3 gave IC$_{50}$ values of 22 ± 0.4 nM, 17 ± 1 nM and 300 ± 45 nM, respectively.[13] When comparing the enantiomers (2*S*,3*S*)-**5** and (2*R*,3*R*)-**5** (both enantioenriched to the same degree, er 7:3) it appears that (2*S*,3*S*)-**5** is only twice as potent an

inhibitor of EAAT3 as (2*R*,3*R*)-**5**, but has very similar inhibitory activity at EAAT1 and EAAT2 compared to its enantiomer. Interestingly, the racemic mixture (50:50) of both 2,3-*syn* (*threo*) enantiomers (*rac*-(2*RS*,3*RS*)-**5**) gave very similar IC$_{50}$ values compared to commercially available (*S*,*S*)-TFB-TBOA. This lack of stereoselectivity in inhibitor binding by EAAT1 and EAAT2 might seem unusual but is not unprecedented for transporters. Poly specific binding of enantiomeric cyclic peptide inhibitors was also observed in the case of the ABC transporter P-glycoprotein (P-gp) due to mainly hydrophobic and aromatic interactions between the inhibitor and the transporter.[32]

The stereoselectivity in terms of inhibitor activity is more pronounced for the 2,3-*anti* (*erythro*, TFB-EBOA) isomers of **5**, in particular in the case of EAAT3; the (2*R*,3*S*)-isomer of **5** was about five times more active than its enantiomer (2*S*,3*R*)-**5**. Furthermore, IC$_{50}$ values for the racemic mixture of both TFB-EBOA enantiomers lay between values of the two individual enantiomers. As for the 2,3-*syn* (*threo*) isomers above, the stereoselection of EAAT1 and EAAT2 for the 2,3-*syn* (*erythro*) isomers was weakly manifested.

Both photoaffinity probes **15** and **16** inhibited transport of [^{3}H]-L-glutamate via EAAT1−3 in a dose-dependent manner (Figure S1). Inhibition of EAAT1 was significantly stronger by **15** than **16** (IC$_{50}$ 0.11 ± 0.02 μM and 1.23 ± 0.29 μM, respectively). Similarly, diazirine **15** was the slightly more active inhibitor than **16** at EAAT2 (IC$_{50}$ 0.40 ± 0.03 μM and 0.82 ± 0.08 μM, respectively), however, for EAAT3 this was the opposite (**15**: IC$_{50}$ 4.03 ± 0.80 μM; **16**: IC$_{50}$ 2.11 ± 0.58 μM). Overall, both photoaffinity probes are more potent inhibitors than the standardly used DL-TBOA (IC$_{50}$ values 48.0 ± 4.1, 7.0 ± 1.1 and 8.0 ± 1.9 μM for EAAT1, EAAT2 and EAAT3, respectively).[11]

CONCLUSIONS

We have developed a short and concise synthesis for the preparation of all four stereoisomers of TFB-TBOA, a known and potent inhibitor of glutamate uptake by excitatory amino acid transporters EAAT1−3. Our only four-step synthetic route relies on the Sharpless asymmetric aminohydroxylation to access the 2,3-*syn* (*threo*) isomers, while we have employed the Davis asymmetric α-hydroxylation to prepare the 2,3-*anti* (*erythro*) isomers of TFB-TBOA. For the first time, all four stereoisomers have been tested on human glutamate transporters EAAT1−3 and our data show that the correct relative stereochemistry is crucial for high inhibitory activity, with the 2,3-*syn* (*threo*) isomers being clearly more active than the 2,3-*anti* (*erythro*) isomers. On the other hand, the absolute stereochemistry plays a minor role and enantioselectivity was effectively only observed for EAAT3. This observation implies that it is probably not necessary to use enantiomerically pure (*S,S*)-TFB-TBOA for biological studies but the racemic mixture, that is very easily synthesized following our developed protocol, is equally potent. Following our synthetic strategy, two TFB-TBOA-derived photoaffinity probes have been synthesized which exhibited submicromolar to micromolar inhibitory activity at EAAT1−3 and therefore could be useful molecular tools for photo-cross-linking studies. Efforts to develop further EAAT molecular probes with improved activities are underway in our laboratory and will be reported in due course.

METHODS

General Procedure for the Synthesis of 2,3-*syn*-β-Hydroxyaspartates (2*S*,3*R*)-10 and (2*R*,3*R*)-10. OsO$_4$ (5 μmol) was added as an aqueous stock solution (61 μL of 2% OsO$_4$ in H$_2$O) to a stirred solution of either (DHQD)$_2$PHAL (14.5 μmol, for obtaining the *S,S*-isomer) or (DHQ)2PHAL (for obtaining the *R,R*-isomer) and 4-chlorobenzoyloxycarbamate **11** (0.438 mmol; for synthesis details, see the Supporting Information) in MeCN (2.5 mL). After stirring at room temperature for 12−25 min, the yellow-brown reaction mixture was cooled to −15 °C and a solution of fumarate **9** (0.438 mmol; for synthesis details, see the Supporting Information) in MeCN/H$_2$O 4:1 (2.5 mL) was added slowly over a period of 15−45 min at −15 °C. Upon addition, the color of the reaction mixture changed to dark green. After completed addition, it was left to warm up to room temperature, whereupon the color changed again to pale yellow. Reaction control revealed no more product formation after 4 h. The reaction was quenched by addition of sat. aqueous K$_2$S$_2$O$_5$ soln. (1 mL) which resulted in the precipitation of a fine brown powder. After addition of H$_2$O (20 mL) and EtOAc (20 mL), the phases were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with sat. aqueous NaHCO$_3$ solution (2 × 15 mL) and brine (15 mL), dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to afford the crude product. This was purified by flash column chromatography (silica gel, EtOAc/hexane 1:9) and gave

syn-β-hydroxyaspartate as single diastereomer. For spectral data of (2*S*,3*S*)-**10** and (2*R*,3*R*)-**10**, see the Supporting Information.

General Procedure for the Synthesis of 2,3-*anti*-β-Hydroxyaspartates (2*S*,3*R*)-10 and (2*R*,3*S*)-10. A solution of *N*-Boc protected (*S*)- or (*R*)-aspartate diester **13** (0.87 mmol) in dry THF (30 mL) was cooled to −78 °C and a solution of NaHMDS in THF (1M, 2.17 mL, 2.17 mmol) was added dropwise. After stirring for 2 h at −78 °C a solution of oxazirine **14** (1.04 mmol; for synthesis details, see the Supporting Information) in dry THF (6 mL) was added at −78 °C to the reaction mixture. The progress of the reaction was monitored by TLC (silica gel, EtOAc/hexane 2:8) and was complete after 30−45 min. The reaction was quenched at −78 °C by addition of a solution of camphorsulfonic acid (3.6 mmol) in THF (3.6 mL) and further diluted with EtOAc (100 mL). The organic phase was washed with aqueous HCl (10%, 100 mL), sat. aqueous NaHCO$_3$ solution (100 mL) and brine (100 mL), dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. The diastereomeric purity of the crude products (determined by ^{1}H NMR) was dr 92:8−94:6. The crude was purified twice by flash column chromatography (silica gel, first column: CH$_2$Cl$_2$/ MeOH 99:1, second column EtOAc/hexane 1:9) to afford *anti*-β-hydroxyaspartate as single diastereomer. For spectral data of (2*S*,3*R*)-**10** and (2*R*,3*S*)-**10**, see the Supporting Information.

ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00311.

Synthesis procedures and spectral data for synthetic intermediates and final compounds, purity assessment of final compounds and details of biological procedures and pharmacological evaluation (PDF)

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Author Contributions

M.L.: developed novel synthetic route, synthesized and characterized intermediate and target compounds, and contributed to writing of manuscript. A.R.: synthesized and characterized intermediate and target compounds. A.S.: generated stable cell lines, performed pharmacological assays, data analysis, and contributed to writing of manuscript. M.H.: supervised the pharmacological analysis. M.L.: supervised the chemistry, designed experiments, and wrote the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ALS, amyotrophic lateral sclerosis; DIPC, *N,N'*-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; EAAT, excitatory

amino acid transporter; EBOA, *erythro-β*-benzyloxyaspartate, (2,3-*anti*)-2-amino-3-(benzyloxy)succinic acid; HMDS, hexamethyldisilazane; SLC, solute carrier (group of membrane transporter proteins); TBOA, *threo-β*-benzyloxyaspartate, (2,3-*syn*)-2-amino-3-(benzyloxy)succinic acid; TFA, trifluoroacetic acid; TFB-TBOA, (2,3-*syn*)-2-amino-3-((3-(4-(trifluoromethyl)benzamido)benzyl)oxy)succinic acid

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