Rapid chemoenzymatic route to glutamate transporter inhibitor L-TFB-TBOA and related amino acids

Fu, Haigen; Younes, Sabry H. H.; Saifuddin, Mohammad; Tepper, Pieter G.; Zhang, Jielin; Keller, Erik; Heeres, Andre; Szymanski, Wiktor; Poelarends, Gerrit J.

Published in:
Organic & Biomolecular Chemistry

DOI:
10.1039/c7ob00305f

Publication date:
2017

Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2017, **15**, 2341

Received 8th February 2017,
Accepted 16th February 2017

DOI: 10.1039/c7ob00305f

rsc.li/obc

Rapid chemoenzymatic route to glutamate transporter inhibitor L-TFB-TBOA and related amino acids†

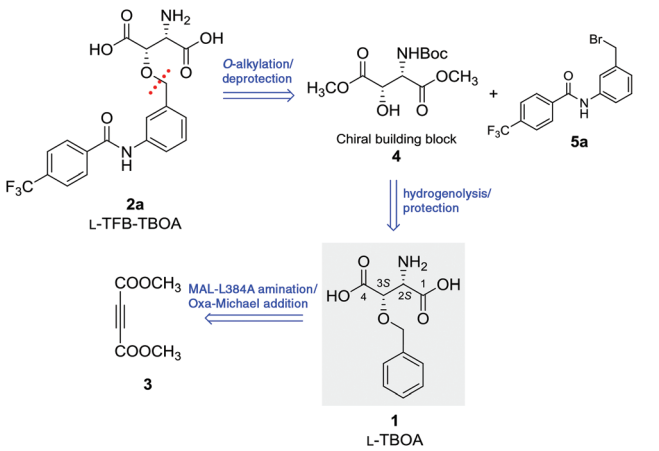
Haigen Fu,‡[a] Sabry H. H. Younes,‡[a] Mohammad Saifuddin,[a] Pieter G. Tepper,[a] Jielin Zhang,[a] Erik Keller,[b] André Heeres,[b] Wiktor Szymanski[c] and Gerrit J. Poelarends*[a]

The complex amino acid (L-*threo*)-3-[3-[4-(trifluoromethyl) benzoylamino]benzyloxy]aspartate (L-TFB-TBOA) and its derivatives are privileged compounds for studying the roles of excitatory amino acid transporters (EAATs) in regulation of glutamatergic neurotransmission, animal behavior, and in the pathogenesis of neurological diseases. The wide-spread use of L-TFB-TBOA stems from its high potency of EAAT inhibition and the lack of off-target binding to glutamate receptors. However, one of the main challenges in the evaluation of L-TFB-TBOA and its derivatives is the laborious synthesis of these compounds in stereoisomerically pure form. Here, we report an efficient and step-economic chemoenzymatic route that gives access to enantio- and diastereopure L-TFB-TBOA and its derivatives at multigram scale.

Introduction

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS) and mediates numerous neuronal communications in the brain.[1] However, accumulation of high levels of extracellular glutamate may lead to over-activation of glutamate-gated ion channels and, consequently, neuronal injury.[2] Synaptic glutamate concentrations are strictly kept below levels of neurotoxicity by the family of excitatory amino acid transporters (EAATs) expressed on neurons and surrounding glial cells.[3] Dysfunction of EAATs has been implicated in many neurological disorders, such as Alzheimer's disease, epilepsy, amyotrophic lateral sclerosis, and Huntington's disease.[4]

L-Aspartate derivatives with aryloxy substituents at the C3 position, exemplified by L-*threo*-3-benzyloxyaspartate (**1**, L-TBOA, Scheme 1), were identified as the first class of nontransportable EAAT inhibitors.[5] The importance of these L-aspartate derivatives as tools in neurobiological research was further highlighted by the identification of the most potent and widely used blocker, (L-*threo*)-3-[3-[4-(trifluoromethyl) benzoylamino]benzyloxy]aspartate (**2a**, L-TFB-TBOA, Scheme 1), which has nanomolar affinity to EAAT1 and EAAT2 and lacks affinity towards glutamate-gated ion channels.[6] However, the asymmetric synthesis of enantiopure L-TFB-TBOA and related compounds proved to be extremely challenging. Shimamoto and coworkers reported the asymmetric synthesis of enantiopure **2a** through an elaborate 20-step synthetic procedure.[7] Although a concise synthesis of **2a** based on Sharpless aminohydroxylation with chiral ligand $(DHQD)_2PHAL$ was recently reported by Leuenberger *et al.*, this



Scheme 1 Chemoenzymatic retrosynthesis of L-TFB-TBOA (**2a**).

[a]*Department of Chemical and Pharmaceutical Biology, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. E-mail: g.j.poelarends@rug.nl; http://www.rug.nl/staff/g.j.poelarends/*
[b]*Syncom BV, Kadijk 3, 9747 AT Groningen, The Netherlands*
[c]*Department of Radiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c7ob00305f

‡These authors contributed equally to this work.

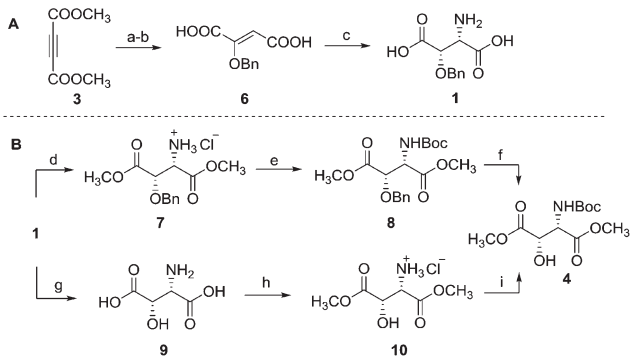
procedure provided **2a** with only 40% enantiomeric excess (ee).[8] Efficient asymmetric synthesis of enantiopure L-TFB-TBOA and its derivatives would facilitate the further discovery of EAAT subtype-selective inhibitors, which are highly useful tools for the elucidation of the exact physiological roles of distinct EAATs in glutamate accumulation,[9] synaptic transmission[6c] and animal behavior.[6a] Hence, alternative procedures that provide efficient and more step-economic access to **2a** and related compounds are in great demand. Herein, we report a rapid chemoenzymatic route that gives convenient access to enantiopure L-TFB-TBOA and its derivatives at multigram scale. This method uses the late functionalization of a common precursor to provide a convenient way of divergent preparation of L-aspartate derivatives with large aryloxy substituents at the C3 position (Scheme 1).

Results and discussion

Retrosynthetic analysis suggests that L-TFB-TBOA (**2a**) could be derived from dimethyl (L-*threo*)-*N*-Boc-3-hydroxyaspartate (**4**) and substituted benzyl bromide **5a** *via* *O*-alkylation and subsequent deprotection (Scheme 1). The key challenge was the formation of chiral building block **4** due to the possible difficulties in constructing the required L-*threo* configuration at vicinal chiral centers with a 1,2-aminoalcohol motif.[10] We envisioned that this key precursor **4** could be readily generated from **1**, which has the desired L-*threo* configuration, *via* protection and hydrogenolysis steps. While the chemical synthesis of **1** is a highly challenging 11-step procedure,[5c] a straightforward three-step chemoenzymatic methodology for the asymmetric synthesis of **1** (de >99%, ee >99%), starting from commercially available dimethyl acetylenedicarboxylate **3**, has recently been reported (Scheme 2A).[11]

To enable efficient multigram-scale (see ESI†) synthesis of **1**, we first optimized the previously used procedure for the small-scale (150 mg) synthesis of **1**.[11b] The addition of benzyl alcohol to **3** yields a mixture of *cis* and *trans* product isomers (Scheme 2A, step a). After ester hydrolysis of this isomeric mixture (step b), we purified the *trans*-2-benzyloxyfumaric acid (**6**) by recrystallization (37% yield over two steps). This provided a higher yield of **6** when compared to the previously reported column chromatography method for isomer separation.[11b] In addition, the efficiency of the methylaspartate ammonia lyase (MAL-L384A)-catalyzed amination of **6** (step c) was enhanced by using NH_3 (instead of NH_4Cl) and pH 9.5 (instead of pH 9.0), leading to >98% conversion and affording product **1** in 80% isolated yield.

Starting from compound **1**, the synthesis of key intermediate **4** was achieved in three successive reactions (steps d–f in Scheme 2B). Diesterification of **1** with $SOCl_2$ in dry methanol, followed by Boc-protection, delivered compound **8** without the need for purification. Hydrogenolysis of **8** using $HCOONH_4$/Pd gave **4** in excellent yield (71% over three steps).[12] Notably, exhaustive esterification of both carboxyl groups of compound **1** needs high $SOCl_2$ concentration (10 equivalents) under

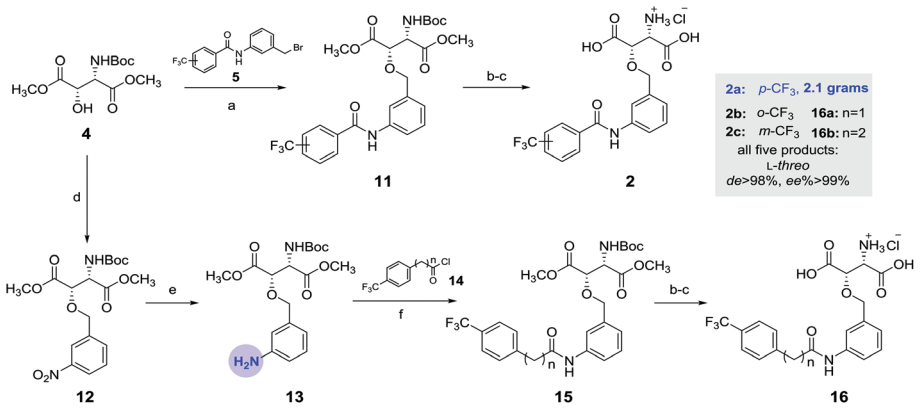


Scheme 2 Chemoenzymatic synthesis of L-TBOA (**1**) and chiral building block **4**. Reagents and conditions: (a) BnOH, DABCO, DCM, rt, 4 h; (b) NaOH (2 M), reflux, 2 h, then HCl (1 M), two-step yield after recrystallization was 37%; (c) MAL-L384A (0.01 mol%), 5 M NH_3/NH_4Cl, 20 mM $MgCl_2$, pH = 9.5, 24 h, conversion >98%, isolated yield 80%; (d) $SOCl_2$, MeOH, reflux, 6 h; (e) di-*tert*-butyl dicarbonate, DIEA, DCM, rt, 24 h; (f) Pd/C, $HCOONH_4$, MeOH, reflux, 45 min, 71% for 3 steps; (g) Pd/C, $HCOONH_4$, MeOH, reflux, 45 min; (h) $SOCl_2$, MeOH, reflux, 6 h; (i) di-*tert*-butyl dicarbonate, DIEA, DCM, rt, 24 h, 73% for 3 steps. DABCO, 1,4-diazabicyclo[2.2.2]octane; DIEA, *N*,*N*-diisopropylethylamine; DCM, dichloromethane.

reflux, while mono-esterification of the C4-carboxyl group of **1** could be easily accomplished under ambient conditions with one equivalent of $SOCl_2$. Another route (steps g–i in Scheme 2B) for preparing the chiral building block **4** was also designed by rearranging the three reactions described above. We initiated the synthetic procedure with debenzylation of **1**, which provided 3-hydroxyaspartic acid (**9**), and which was followed by esterification and Boc-protection to afford **4** in three steps with 73% overall yield.

With chiral building block **4** in hand, the synthesis of target molecule L-TFB-TBOA (**2a**) could be accomplished through *O*-alkylation, followed by global deprotection (steps a–c in Scheme 3). This strategy provides a general synthesis route towards derivatives of L-*threo*-3-hydroxyaspartic acid, including valuable analogs of L-TBOA and L-TFB-TBOA. To facilitate efficient *O*-alkylation, which is effected by a nucleophilic substitution reaction of **4** and **5** (for synthesis details, see ESI†), the strong base NaH was used to deprotonate the hydroxyl group of **4**. A low temperature (−20 °C) was needed to avoid epimerization, and the desired compound **11** (*i.e.*, globally protected **2a**) was obtained with an isolated yield of 45%. Subsequently, global deprotection of **11** was conducted *via* treatment with TFA and followed by hydrolysis with LiOH, providing the desired final product **2a** (L-TFB-TBOA hydrochloride) in a yield of 59% over two deprotection steps.

As anticipated, product **2a** was identified as the desired *threo* isomer (de >98%) by comparison of its ^{1}H-NMR signals and *J*-coupling values to those of an authentic standard (commercially available L-TFB-TBOA) and chemically synthesized DL-*threo* and DL-*erythro* stereoisomers (Table S1†). To determine the absolute configuration of product **2a**, chiral HPLC analysis was conducted by using the authentic standard L-TFB-TBOA and chemically synthesized DL-TFB-TBOA (see ESI†) as refer-

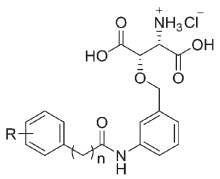


Scheme 3 Synthesis of L-TFB-TBOA and its derivatives. Reagents and conditions: (a) $ArCH_2Br$ (**5**, see ESI†), NaH, DMF, −20 °C, 4 h, 42%–50%; (b) TFA/DCM (2 : 5, v/v), 0 °C, 1.5 h; (c) THF/H_2O (1 : 1, v/v), LiOH, rt, 2 h, then HCl (1 M), 38%–59%; (d) 3-nitrobenzyl bromide, NaH, DMF, −20 °C, 4 h, 61%; (e) Pd/C, H_2, MeOH, 25 min, 92%; (f) acyl chlorides (**14**, see ESI†), TEA, DCM, rt, 2 h, 51%–65%. TFA, trifluoroacetic acid; THF, tetrahydrofuran; TEA, trimethylamine.

ence molecules. This analysis revealed that the chemoenzymatically produced **2a** is present as a single enantiomer with exclusively the L-*threo* configuration (ee >99%, Table 1). The usefulness of our synthesis strategy was further demonstrated by the preparation of optically pure **2a** at multigram-scale (2.1 g; see ESI†). In addition, two novel optically pure L-TFB-TBOA analogs, **2b** (*o*-CF_3, de >98%, ee >99%) and **2c** (*m*-CF_3, de >98%, ee >99%), were prepared using this newly developed methodology (Table 1).

Medicinal chemists are highly interested in building a large collection of *meta*-substituted analogs of L-TBOA to screen for potent and selective inhibitors of EAAT subtypes.[6b,13] In order to rapidly construct a library of L-TBOA analogs with various groups at the *meta*-position, we envisioned that intermediate **13**, with a free *m*-NH_2 group, would be a convenient precursor for fast structural diversification by applying combinatorial chemistry methodologies. The synthesis of compound **13** was accomplished through a nucleophilic substitution reaction between **4** and 3-nitrobenzyl bromide, yielding **12**, followed by conversion of the nitro group to an amino group with Pd/C/H_2, in a yield of 56% over two steps. To demonstrate the utility of intermediate **13**, we synthesized two L-TFB-TBOA analogs (**16a–b**) with a longer alkyl spacer between the two phenyl rings (Scheme 3). Compound **15** was formed *via* amidation of the amino group of **13** with acyl chloride **14**. Target products **16a** and **16b** were obtained in optically pure form after two steps of deprotection (Table 1).

Table 1 Absolute configuration of chemoenzymatically obtained L-TFB-TBOA and its derivatives



Entry	No.	R	*n*	de[a] [%]	ee[b] [%]	Absolute configuration
1	**2a**	*p*-CF_3	0	>98	>99	(2*S*,3*S*)[c]
2	**2b**	*o*-CF_3	0	>98	>99	(2*S*,3*S*)
3	**2c**	*m*-CF_3	0	>98	>99	(2*S*,3*S*)
4	**16a**	*p*-CF_3	1	>98	>99	(2*S*,3*S*)
5	**16b**	*p*-CF_3	2	>98	>99	(2*S*,3*S*)

[a] Diastereomeric excess (de) was determined by ^{1}H NMR. [b] Enantiomeric excess (ee) was determined by chiral HPLC. [c] Absolute configuration was determined unambiguously by comparison of ^{1}H NMR, chiral HPLC and optical rotation data to those of an authentic sample of L-TFB-TBOA (2*S*,3*S*).

Conclusion

In conclusion, we have managed to construct the complex amino acid L-TFB-TBOA using only 9 steps with 6% overall yield, starting from commercially available dimethyl acetylenedicarboxylate. Compared with the previously reported 20-step synthesis of L-TFB-TBOA, this is a dramatic reduction in step count with fewer than half the steps. This chemoenzymatic synthesis methodology can be easily up-scaled to multigram-scale and gives convenient access to enantiopure derivatives of L-TBOA and L-TFB-TBOA.

Experimental section

For detailed experimental procedures and characterization of compounds, see ESI.†

Acknowledgements

This research was financially supported by the Netherlands Organisation for Scientific Research (KIEM grants 731.013.110 and 731.015.108). Haigen Fu and Jielin Zhang acknowledge funding from the China Scholarship Council. The authors thank Dr Marianne de Villiers for insightful discussions.

Notes and references

1 (*a*) N. C. Danbolt, *Prog. Neurobiol.*, 2001, **65**, 1; (*b*) P. Beart and R. O'shea, *Br. J. Pharmacol.*, 2007, **150**, 5; (*c*) R. J. Vandenberg and R. M. Ryan, *Physiol. Rev.*, 2013, **93**, 1621; (*d*) A. A. Jensen, C. Fahlke, W. E. Bjørn-Yoshimoto and L. Bunch, *Curr. Opin. Pharmacol.*, 2015, **20**, 116.

2 E. E. Benarroch, *Neurology*, 2010, **74**, 259.

3 A. V. Tzingounis and J. I. Wadiche, *Nat. Rev. Neurosci.*, 2007, **8**, 935.

4 L. Bunch, M. N. Erichsen and A. A. Jensen, *Expert Opin. Ther. Targets*, 2009, **13**, 719.

5 (*a*) B. Lebrun, M. Sakaitani, K. Shimamoto, Y. Yasuda-Kamatani and T. Nakajima, *J. Biol. Chem.*, 1997, **272**, 20336; (*b*) K. Shimamoto, B. Lebrun, Y. Yasuda-Kamatani, M. Sakaitani, Y. Shigeri, N. Yumoto and T. Nakajima, *Mol. Pharmacol.*, 1998, **53**, 195; (*c*) K. Shimamoto, Y. Shigeri, Y. Yasuda-Kamatani, B. Lebrun, N. Yumoto and T. Nakajima, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2407.

6 (*a*) K. Shimamoto, R. Sakai, K. Takaoka, N. Yumoto, T. Nakajima, S. G. Amara and Y. Shigeri, *Mol. Pharmacol.*, 2004, **65**, 1008; (*b*) K. Shimamoto, *Chem. Rec.*, 2008, **8**, 182; (*c*) S. Tsukada, M. Iino, Y. Takayasu, K. Shimamoto and S. Ozawa, *Neuropharmacology*, 2005, **48**, 479.

7 K. Shimamoto, *WO* 2003/000698A000691, 2003.

8 M. Leuenberger, A. Ritler, A. Simonin, M. A. Hediger and M. Lochner, *ACS Chem. Neurosci.*, 2016, **7**, 534.

9 D. Jabaudon, K. Shimamoto, Y. Yasuda-Kamatani, M. Scanziani, B. Gähwiler and U. Gerber, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 8733.

10 (*a*) D. L. Boger, R. J. Lee, P.-Y. Bounaud and P. Meier, *J. Org. Chem.*, 2000, **65**, 6770; (*b*) L. Harris, S. P. Mee, R. H. Furneaux, G. J. Gainsford and A. Luxenburger, *J. Org. Chem.*, 2010, **76**, 358; (*c*) Y. Jiang, X. Chen, Y. Zheng, Z. Xue, C. Shu, W. Yuan and X. Zhang, *Angew. Chem., Int. Ed.*, 2011, **50**, 7304; (*d*) A. C. Willis and M. D. McLeod, *J. Org. Chem.*, 2012, **77**, 8480; (*e*) D.-F. Lu, C.-L. Zhu, Z.-X. Jia and H. Xu, *J. Am. Chem. Soc.*, 2014, **136**, 13186.

11 (*a*) H. Raj, W. Szymański, J. de Villiers, H. J. Rozeboom, V. P. Veetil, C. R. Reis, M. de Villiers, F. J. Dekker, S. de Wildeman, W. J. Quax, A.-M. W. H. Thunnissen, B. L. Feringa, D. B. Janssen and G. J. Poelarends, *Nat. Chem.*, 2012, **4**, 478; (*b*) J. de Villiers, M. de Villiers, E. M. Geertsema, H. Raj and G. J. Poelarends, *ChemCatChem*, 2015, **7**, 1931.

12 T. Bieg and W. Szeja, *Synthesis*, 1985, 76.

13 J. C. Hansen, W. E. Bjørn-Yoshimoto, N. Bisballe, B. Nielsen, A. A. Jensen and L. Bunch, *J. Med. Chem.*, 2016, **59**, 8771.