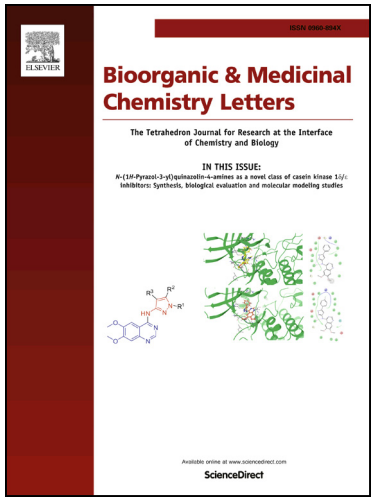

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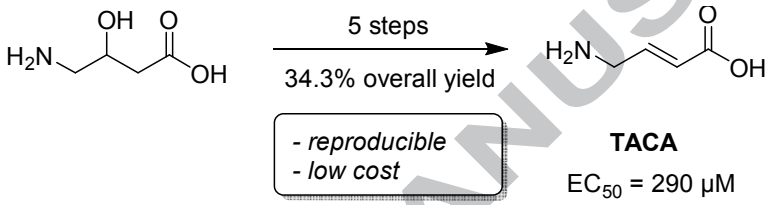

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ABSTRACT

Investigations into the pharmacology of different types of cys-loop GABA receptor have relied for years on the chemical modification of GABA-like compounds. The GABA metabolite GABOB is an attractive molecule to modify due to its convenient chemical structure. In the process of developing new GABA-mimic compounds from GABOB as a starting compound three small molecule GABA derivatives were synthesized using a variety of chemical transformations. Amongst these, a new and reliable method to synthesize TACA (trans-4-aminocrotonic acid) is reported.

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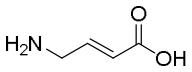
Investigations into the function and pharmacology of cys-loop GABA receptors in both mammalian and invertebrates systems have relied, in part, on their sensitivity to various agonists and synthesized GABA derivatives.[1-3] The conformationally restricted analogue of GABA, trans-4-aminocrotonic acid (TACA) (Fig. 1) has been studied extensively and used to distinguish different GABA receptor subtypes.[4] While now available commercially at a high cost (ca. $1000/100mg), TACA was originally prepared through the dehydration of 4-amino-3-hydroxybutyric acid (GABOB).[5] Since then there have been no published reports of alternative approaches for the synthesis of TACA from GABOB. However, numerous reports continue to utilize TACA as a pharmacological tool in various receptor studies.[6-9] As such, this study presents a new and reliable method to synthesize TACA from GABOB at an overall cost of approximately $25/100mg.

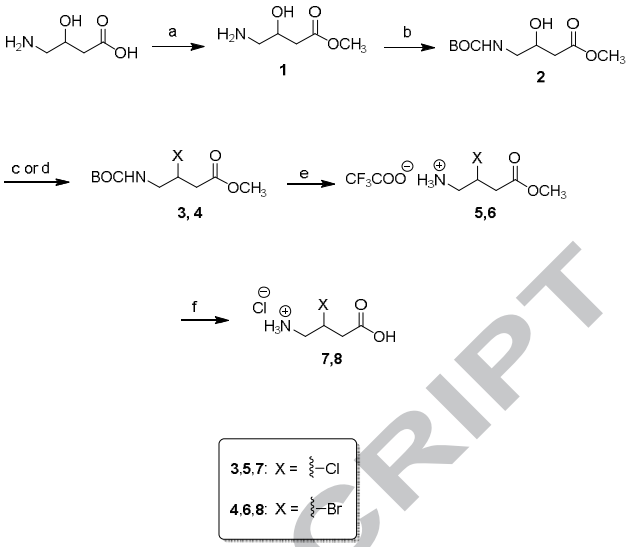
Given that both stereoisomers of 4-amino-3-hydroxybutyric acid (*R*(–)-GABOB and *S*(+)-GABOB) are ligands for the receptor, we sought to also examine whether other closely related electronic isostere derivatives would make suitable substrates. The hydroxyl group in both stereoisomers of GABOB is electronegative. Therefore, we wondered whether other electronegative derivatives would serve as suitable substrates. As such, we decided to synthesize halogenated GABA derivatives with chlorine or bromine substitution at the C3 position.

Commercially available racemic GABOB was converted to a methyl ester derivative **1** by reaction with trimethylsilyl chloride and anhydrous methanol in quantitative yield.[10] Subsequently, the amino group was protected with the *tert*-butoxycarbonyl group, by reacting **1** with di-*tert*-butyl dicarbonate (BOC_2O) in basic solution to afford **2** in good yield. Substitution of the hydroxyl group of **2** with trichloroacetonitrile and triphenylphosphine afforded the chlorinated derivative **3** in 65% yield, whereas reaction of **2** with triphenylphosphine with carbon tetrabromide afforded the brominated derivative **4** in 75% yield. Finally, deprotection of the BOC group, first with trifluoroacetic acid, followed by ester hydrolysis with a 2M HCl solution under reflux, afforded the halogenated derivatives **7** and **8** in excellent yield (Scheme 1).



TACA

Figure 1. Structure of TACA

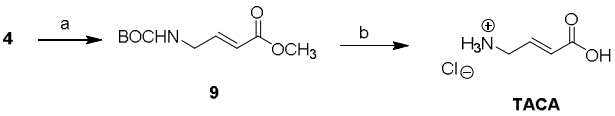


Conditions: (a) 2 equiv of trimethylsilyl chloride (TMSCl), methanol, r.t., 100%; (b) di-*tert*-butyl dicarbonate (BOC_2O), $NaHCO_3$, THF, water, 24 hr, 76%; (c) 3 equiv of triphenylphosphine, 1.5 equiv of trichloroacetonitrile, toluene, 70 °C, 15 min, 65% of **3**; (d) 2.0 equiv of triphenylphosphine, 2.0 equiv of carbon tetrabromide, toluene, r.t., 1 hr, 75% (e) TFA, DCM, r.t., 82% (**5**) or 98% (**6**); (f) 2M HCl, 3 hr, 84% (**7**) or 100% (**8**).

Scheme 1. Synthesis of chlorinated and brominated GABOB derivatives.

TACA is a well characterized GABA receptor agonist.[4] The report describing its synthesis is several decades old and is poorly characterized.[5] Therefore, based on the successful syntheses of the halogenated derivatives **3** and **4**, we sought to expand the synthesis of TACA by subjecting compound **4** to basic conditions, in order to determine if elimination of the chloride group would afford the alkene TACA.

We investigated a variety of bases (potassium *tert*-butoxide, potassium carbonate, triethylamine, and sodium hydride) in the presence of compound **4** to determine which base would be most appropriate to form the TACA derivative. We identified that with three equivalents of K_2CO_3, the allyl compound **9** formed in good yield. Deprotection of both the BOC group, and the methyl ester in refluxing 2M HCl afforded the TACA salt in excellent yield (Scheme 2).



Conditions: (a) 3 equiv of K_2CO_3, DMF:THF = 1:1, r.t., 70%; (b) 2M HCl, 3 h, reflux, 86%.

Scheme 2. Synthesis of TACA

Therefore, TACA can be reliably synthesized in five steps from racemic GABOB in 34.3% overall yield. Only two of the five steps requires column chromatography, so it is feasible to scale-up and synthesize TACA from GABOB in several days. Although this synthesis is more steps compared to the reports from Musashi in 1954,[5] this is a relatively efficient approach providing TACA in a reliably good yield.

To test the activity of compounds TACA, **7** and **8**, we expressed the UNC-49B/C GABA receptor from the parasitic nematode *Haemonchus contortus* in *Xenopus laevis* oocytes and characterized the channel using 2-electrode voltage clamp electrophysiology and procedures outlined in Kaji et al 2015.[9] Compounds **7** and **8** which were tested at 500 µM did not activate the channel. However, TACA did successfully activate the channel with an EC_{50} (290 ± 15 µM; n = 9) which is in a similar range as previously reported for this receptor (Figure 2).[9]

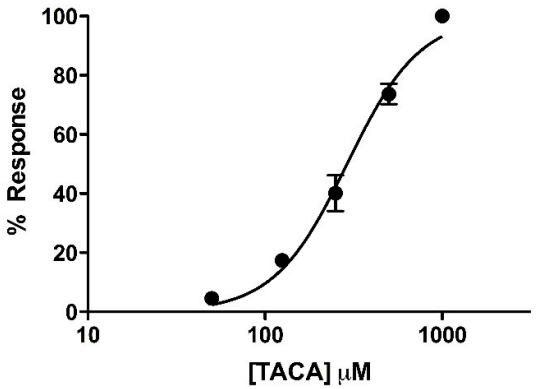


Figure 2: Dose-response analysis of TACA activation at the *H. contortus* UNC-49 receptor

It is interesting to note that the binding site of the UNC-49B/C GABA receptor contains a serine residue at position 215 that appears to interact with the electronegative hydroxyl group of GABOB.[9] The derivatives **7** and **8** which contain electronegative atoms Cl and Br might not be capable of hydrogen bonding due to their larger size and low electron distribution and may explain why these compounds failed to activate the channel.

In conclusion we report a relatively straightforward method of synthesizing TACA from the starting compound GABOB. This method, while requiring more synthetic steps compared to the traditional method, does result in good reproducible yield. This method can also be further modified leading to the synthesis of additional GABA derivatives.

Acknowledgments

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Supplementary Material

Experimental procedures and copies of NMRs are found here

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