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Synthesis of *(S)*-3-amino-4-(difluoromethylenyl)-cyclopent-1-ene-1-carboxylic acid (OV329), a potent inactivator of γ-aminobutyric acid aminotransferase

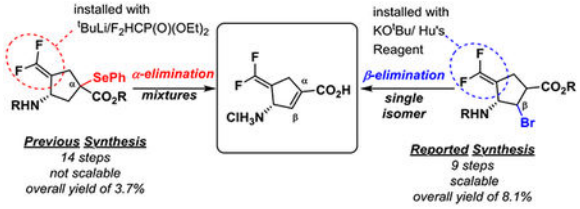
Matthew J. Moschitto and **Richard B. Silverman***

Departments of Chemistry and Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, and Center for Developmental Therapeutics, Northwestern University, Evanston, Illinois 60208, United States

Abstract

(*S*)-3-amino-4-(difluoromethylenyl)cyclopent-1-ene-1-carboxylic acid (OV329, **1**) is being developed for the treatment of addiction and epilepsy. The previous 14-step synthesis of **OV329** was low yielding, involved an unselective α-elimination to form the cyclopentene, required the use of *tert*-butyllithium, and produced toxic selenium by-products in the penultimate step. A new synthesis, which avoids the aforementioned issues, was carried out on large scale, reducing the step count from 14 to 9 steps and increasing the overall yield from 3.7% to 8.1%.

Graphical Abstract



γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system.[1] When GABA concentrations in the brain diminish below a threshold level, convulsions can occur; increasing GABA levels has been shown to stop convulsions and has been clinically indicated for the treatment of epilepsy.[1–3] Additionally, increased concentrations of GABA antagonize the release of dopamine from the nucleus accumbens, a region of the hypothalamus associated with reward and motivation, and has been suggested as a possible treatment of addiction.[4] Unfortunately, systemic administration of GABA is not viable as GABA does not cross the blood brain barrier under ordinary conditions. However, GABA concentrations in the brain can be increased by inhibiting GABA aminotransferase (GABA-AT), a pyridoxal-5'-phosphate (PLP)-dependent enzyme that degrades GABA to succinic semialdehyde. 4-Aminohex-5-enoic acid, also known as vigabatrin (marketed as

***Corresponding Author:** R.B.S.: Tel.: 847-491-5653. Agman@chem.northwestern.edu.

Supporting Information

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Procedures, characterization, spectra (PDF)

Sabril), currently is the only FDA-approved inhibitor of GABA-AT for the treatment of infantile spasms and has been shown to be a possible treatment for addiction.[4–6] However, when vigabatrin is taken in large doses (1–3 g/day), it inhibits multiple GABA receptors/enzymes and, with prolonged use, causes retinal damage in 25–40% of patients.[7]

Because of the drawbacks of vigabatrin, we recently reported the design, synthesis, and evaluation of (*S*)-3-amino-4-(difluoromethylenyl)cyclopent-1-ene-1-carboxylic acid (**OV329**, **1**), a potent inactivator of GABA-AT (Figure 1).[8] **OV329** inhibits GABA-AT via enzyme-catalyzed hydrolysis of the difluoromethenyl group, resulting in dicarboxylate metabolite **2**, which binds tightly to the enzyme via electrostatic interactions. *In vivo* studies in rats indicated that **OV329** was vastly superior to previous inhibitors of GABA-AT at suppressing the release of dopamine in the corpus striatum after exposure to cocaine or nicotine. Additionally, **OV329** does not inhibit off-target aminotransferase enzymes such as alanine aminotransferase and aspartate aminotransferase. It additionally does not inhibit the hERG potassium ion channel or various microsomal cytochrome P450 enzymes. Furthermore, on the basis of the Irwin test, the no observed adverse effect level in mice was 6 mg/kg by oral administration. Given the potency (effective in vivo at 0.01–0.1 mg/kg), lack of off-target activity, low plasma protein binding, high microsome stability, lack of activity against the Cerep SpectrumScreen panel of 176 pharmacological targets, negative Ames test, and promising in vivo pharmacokinetics and toxicology of **1**, we look to further study the possibility of **OV329** as a treatment for addiction and epilepsy.

The major hindrance in moving forward with advanced preclinical studies is straightforward synthetic access to **OV329**. Currently, **OV329** has been synthesized in six steps from CPP-115 (**3**), an inhibitor of GABA-AT that we previously designed, currently in Phase 1 clinical trials for the treatment of epilepsy (Figure 2a).[5,9] Given that CPP-115 requires an 8-step synthesis,[10] the total synthetic step count from commercial material to **OV329** is 14 with an overall yield of 3.7%. The synthesis of CPP-115, itself, involves the use of pyrophoric *tert*-butyllithium (on gram scale) to install the 1,1'-difluoroolefin, which limits the scale at which the reaction can be run. Furthermore, the current synthesis relies of the introduction of the cyclopentene through selenoxide elimination. Protected CPP-115 (not shown) is selenated in 70% yield, although yields can vary depending on scale (Figure 2a). Elimination of the oxidized selenide in **4** yields a mixture of chromatographically inseparable isomers, **1** and **5**, in a 5:3 ratio, favoring **1**. Isomer **5** is then selectively degraded using thiosalicylic acid to produce solely **1** in an overall yield of 36% from **4**. Currently, only small amounts (<20 mg) of **OV329** can be obtained. Additionally, given that selenium is regulated by the FDA to levels below 80–150 μg/day, the production of selenol in the penultimate step complicates the synthesis and future FDA consideration of **OV329**.[11]

We therefore developed a second-generation synthesis that: (a) is scalable and high yielding, (b) avoids the use of selenium and *tert*-butyllithium, and (c) does not form multiple isomers from an α-elimination (Figure 2b). We propose that elimination of a leaving group from the β-position would preclude the possibilty of a mixture of isomers and streamline the synthesis. Thus, an intermediate such as **7** would be desirable. Intermediate **7** would be derived from the hydrolysis of bicyclic compound **8**, which could come from known intermediate **9**.

Starting from the chiral Vince lactam (**10**) and following a slight modification of the literature steps,[12] **11** was obtained in good yield on multi-gram scale (Scheme 1). Methanolysis of the acetate and oxidation yielded ketone **9**. Slight modifications of these steps, such as the use of PMBOH/HCl, allowed us to run them on multi-gram scale. The first key step involved the difluoro-Horner-Wadsworth-Emmons olefination of ketone **9**. The standard conditions, which were identical to those used in the synthesis of CPP-115, of *tert*-BuLi and $F_2CHP(O)(OEt)_2$, did not yield the product.[10] Alteration of temperature, base, and addition method did not produce **13**. A more traditional Wittig reaction with Ph_3PCHF_2Br[13,14] also failed to provide **13**. When Hu's reagent, **12**,[15,16] was employed with KO^{t}Bu as base, following Hu's reported conditions, **13** was obtained in small amounts (<10% yield). Given the complex procedure, a more in-depth optimization was carried out. On the basis of Hu's mechanism of this reaction, multiple intermediates form during the course of the reaction.[15,16] Intermediate **17**, which forms first, rearranges via cyclic intermediate **18** to form sulfonate **19**, which is then protonated, triggering elimination and formation of the olefin (Scheme 2). If the reaction was quenched at −60 °C with 6 M HCl five minutes after the addition of KO^{t}Bu to a mixture of **9** and **12**, then only **17** was observed by LC/MS (entry 1, Table 1). **17** did not rearrange to **13** or **19**, even upon heating to 100 °C. Further heating caused hydrolysis of the lactam. Addition of KO^{t}Bu followed by a 6 M HCl quench at −60 °C (entry 2), and subsequent heating at 60 °C for 1 h provided **13** in 9% yield along with starting material and intermediate **17**. Quenching after 1 hour with a saturated NH_4Cl solution, followed by 6 M HCl, slightly improved the yield (entry 3). We thought that a slow infusion of base would limit degradation of **12**, considering its reported instability even at low temperatures.[17] Slow infusion of base with a syringe pump over 30 min with an NH_4Cl/6 M HCl quench dramatically increased the yield to 45%. Prolonging infusion of base to one hour increased the yield to 58%. The reaction did not seem greatly affected by scale as an outside company was able to scale the reaction to over 100 g without a decrease in yield.

With **13** in hand, the next step was the methanolysis of the lactam and elimination. Deprotection of **13** proceeded smoothly to yield **14** in 80% yield. A small amount of 4-methoxybenzoyl-protected lactam also was isolated. Boc protection of **14** activated the lactam for methanolysis with K_2CO_3 and methanol, leading to subsequent elimination of the bromide. This reaction proceeded smoothly in a 53% yield over two steps with no observable isomerization of the olefin. Final deprotection at 80 °C in 6 M HCl yielded **1** in 97% yield with no observable isomerization or degradation. Overall, the yield from Vince lactam (**10**) to **OV329** was 8.1%. The reaction scheme sequence was repeated with little to no modification by an outside company on kilogram scale resulting in over 40g of **OV329** with a total yield of 3.7%.

In conclusion, we have developed a new method for the synthesis of **OV329** (**1**), a potent inactivator of GABA-AT for the potential treatment of epilepsy and addiction. This method reduces the number of synthetic steps from 14 to 9, while increasing the overall yield for the synthesis from 3.7% to 8.1%. Furthermore, the synthesis does not involve the use of toxic selenium in the penultimate step or the use of *tert*-butyllithium. The elimination to form the cyclopentene is selective resulting in a single isomer, **1**, and the entire synthesis can be run

on kilogram scale. The key step involves the use of Hu's reagent (**12**) to furnish a 1,1'-difluoroalkene followed by methanolysis and subsequent elimination. With an increased amount of **OV329** in hand, we can now move into advanced preclinical studies for the treatment of epilepsy and addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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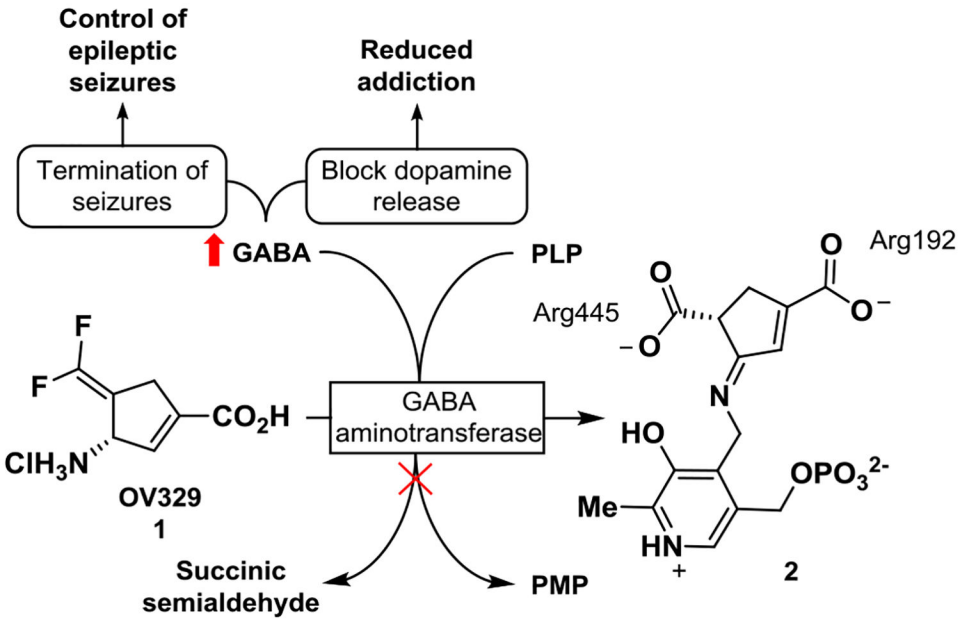


Figure 1.

OV329 (**1**) inhibits GABA-AT through hydrolysis of the 1,1'-difluoromethylene unit, resulting in metabolite **2** and an increase in the concentration of GABA, which is beneficial in the treatment of epilepsy and addiction. PLP: pyridoxal-5'-phosphate; PMP: pyridoxamine-5'-phosphate; GABA: γ-aminobutyric acid.

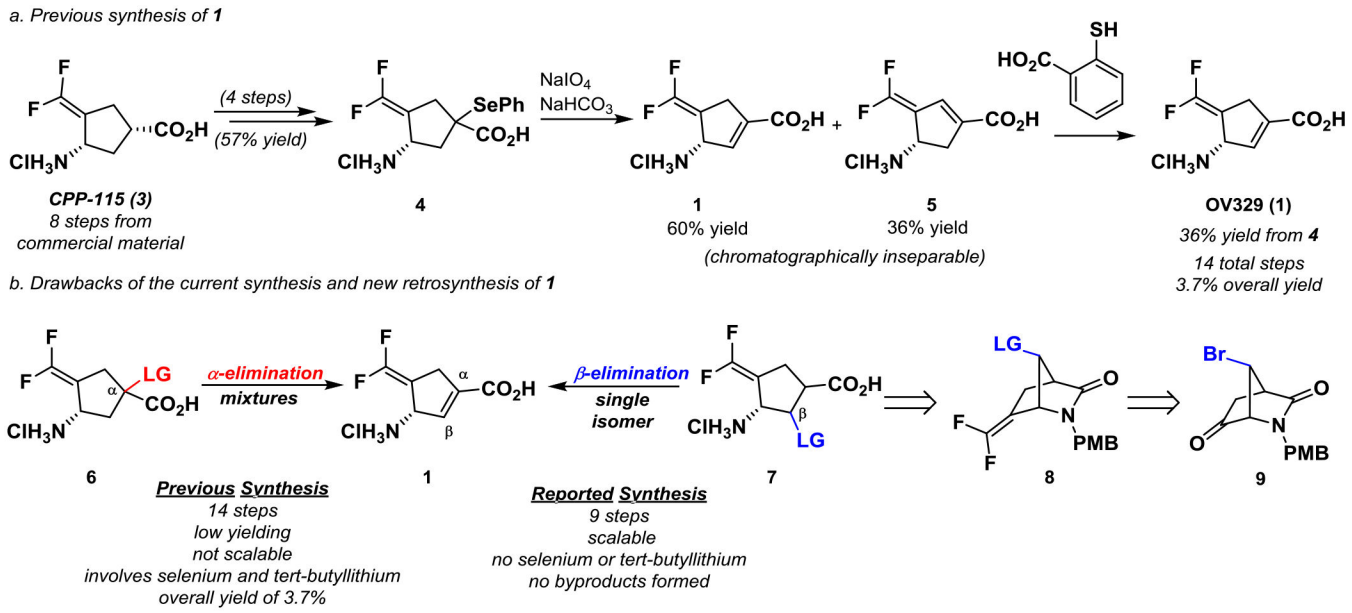
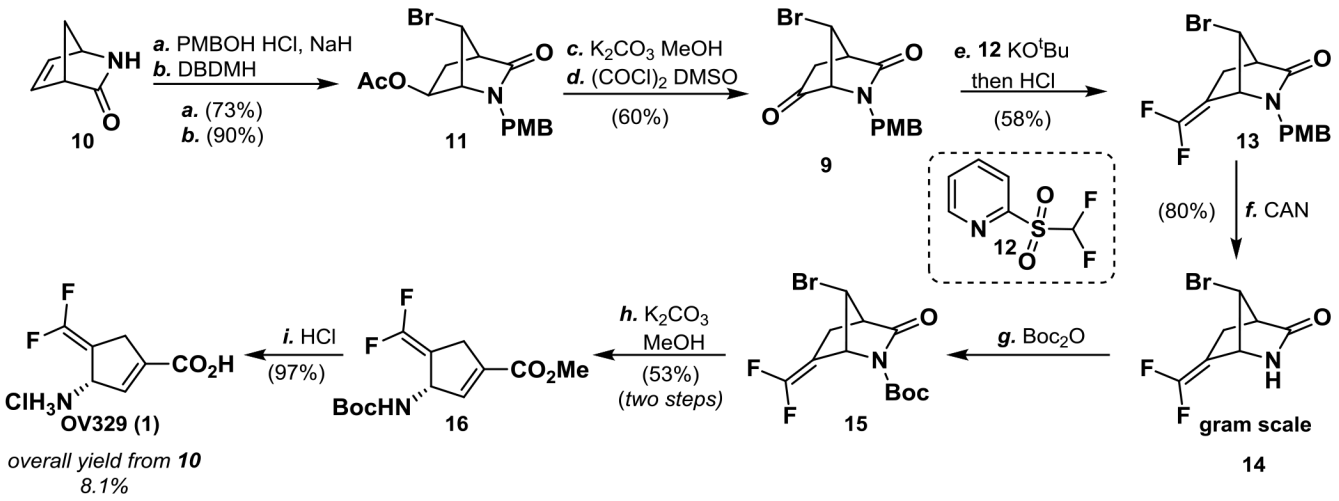


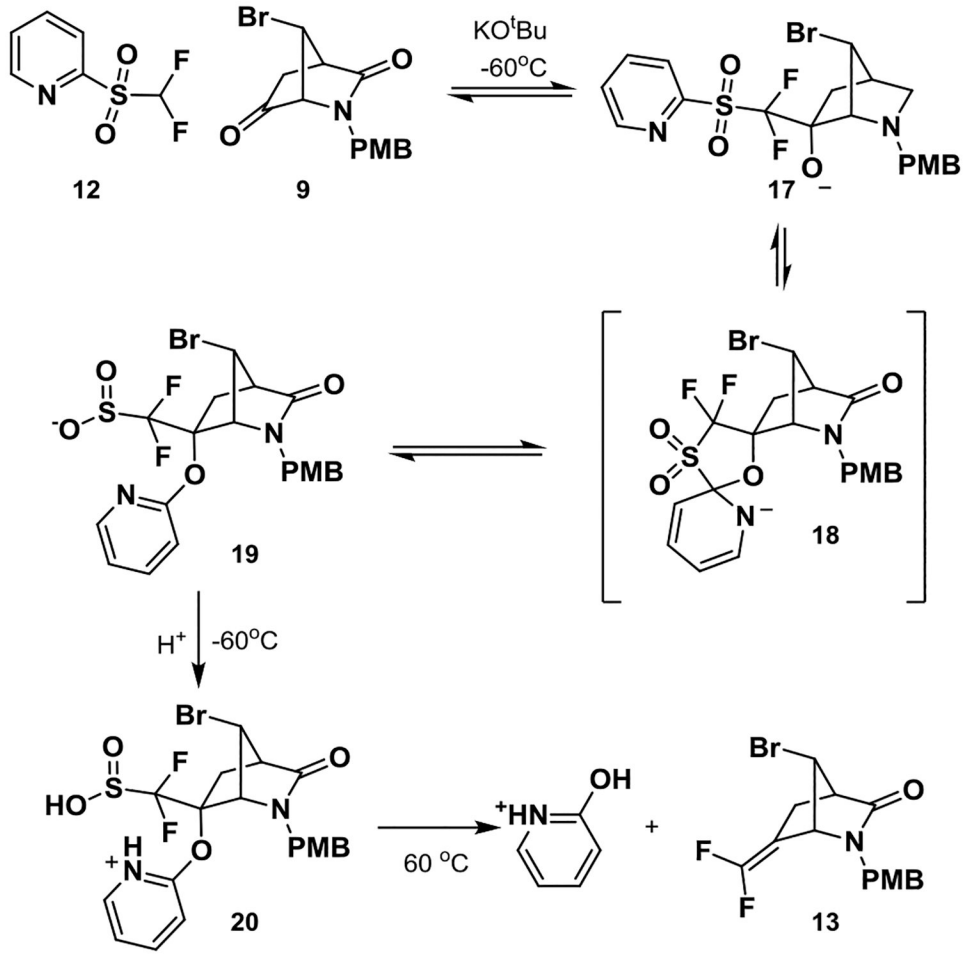
Figure 2.

(a) The previous synthesis of **OV329** (**1**) resulted in a mixture of isomers (**1** and **5**) as the result of an unselective α-elimination of a phenyl selenyl ether. (b) This nonselectively and use of toxic selenium was a major drawback of the current synthesis. A new synthesis, through a β-elimination, whose retrosynthesis is shown, addresses these drawbacks.



Scheme 1: Improved synthesis of OV329.

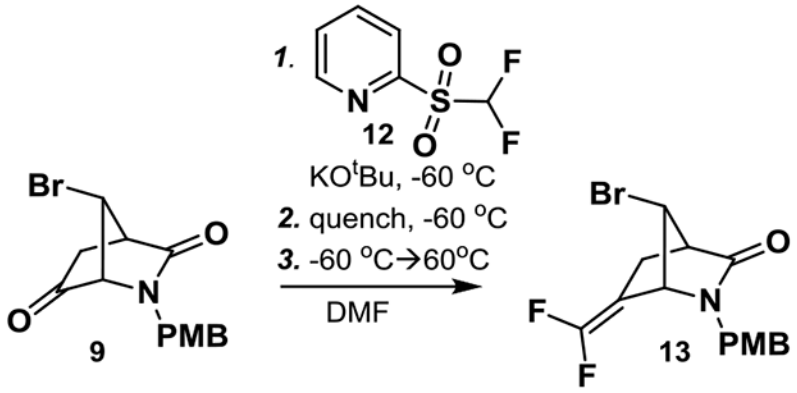
Reagents and conditions: (a) PMBOH (1.5 equiv.), HCl, then **10**, NaH (1.1 equiv.) 0 °C, THF/DMF (1:1), 6 h, 73%; (b) DBDMH (0.6 equiv), AcOH, 23 °C, 6 h, 90%; (c) K_2CO_3 (3 equiv), MeOH 1 h; (d) $(COCl)_2$ (1.4 equiv), DMSO (2.3 equiv), Et_3N (7 equiv), −78 °C, DCM, 60% over two steps; (e) see Table 1, **12** (1.2 equiv), KO^{t}Bu (1.5 equiv), DMF, −60 °C, 30 min, then NH_4Cl, then 6 M HCl, then 23 °C, then 60 °C, 1 h, 58%; (f) CAN (3 equiv), MeCN, H_2O, 0 °C, 1 h, 80%; (g) Boc_2O (1.2 equiv), DMAP (0.1 equiv), Et_3N (1.5 equiv), CH_2Cl_2, 1 h; (h) K_2CO_3 (3 equiv), MeOH, 6 h, 52% over two steps; (i) HCl (6 M), dioxane, 80 °C, 2 h, 97%. Abbreviations: PMBOH: 4-methoxybenzyl alcohol; DMF: *N*-*N*-dimethylformamide; DBDMH: 1,3-dibromo-5,5-dimethylhydantoin; CAN: ceric ammonium nitrite; DMAP: *N*,*N*-dimethylaminopyridine



Scheme 2.
Proposed Mechanism of Fluorination

Table 1

Optimization of Fluorination [a]



entry	Scale (g)	base addition method	quench (time)[b]	yield[c]
1	0.05	dropwise over 5 min	6 M HCl (5 min)	0
2	0.05	dropwise over 5 min	6 M HCl (1 h)	9%
3	0.05	dropwise over 5 min	a. sat. NH_4Cl (1 h) b. 6 M HCl (1 min)	15%
4	0.13	infusion over 30 min	a. sat. NH_4Cl (1 h) b. 6 M HCl (2 min)	45%
5	1	infusion over 60 min	a. sat. NH_4Cl (1 h) b. 6 M HCl (2 min)	58%
6	3.5	infusion over 90 min	a. sat. NH_4Cl (1 h) b. 6 M HCl (2 min)	50%
7	120	dropwise over 400 min	a. sat. NH_4Cl (0.5 h) b. 6 M HCl (10 min)	58%

[a] Conditions: **9** (1 equiv), **12** (1.2 equiv), DMF (0.3 M) −60 °C, then KO^{t}Bu (1.5 equiv) in DMF (0.5 M), then quench at −60 °C, then 23 °C, then 60 °C for 1 h;

[b] time *before* quenching solution was added;

[c] isolated yield after chromatography.