JOC The Journal of Organic Chemistry

pubs.acs.org/joc Article

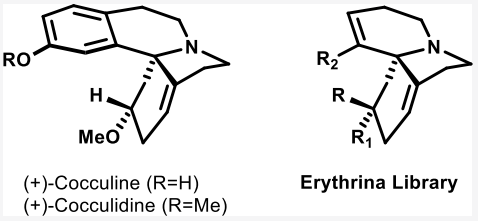
Erythrina Alkaloid Analogues as nAChR Antagonists—A Flexible Platform for Leads in Drug Discovery

Sebastian Clementson, Sergio Armentia Matheu, Emil Märcher Rørsted, Henrik Pedersen, Anders A. Jensen, Rasmus P. Clausen, Paulo Vital, Emil Glibstrup, Mikkel Jessing, and Jesper L. Kristensen*

Cite This: J. Org. Chem. 2021, 86, 8248–8262 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Erythrina alkaloids and their central nervous system effects have been studied for over a century, mainly due to their potent antagonistic actions at β2-containing nicotinic acetylcholine receptors (nAChRs). In the present work, we report a synthetic approach giving access to a diverse set of Erythrina natural product analogues and present the enantioselective total synthesis of (+)-Cocculine and (+)-Cocculidine, both found to be potent antagonists of the β2-containing nAChRs.



INTRODUCTION

Extracts from the seeds of Erythrina plants have been utilized as hypnotics, sedatives, and arrow poison by indigenous South Americans for the past centuries. Many alkaloids of this natural product family possess a curare-like neuromuscular blocking effect, and comprehensive studies in the early 1940s concluded that a majority of the isolated Erythrinanes exert their principal pharmacological activity *via* the central nervous system by acting as competitive antagonists of nicotinic acetylcholine receptors (nAChRs), a heterogeneous class of pentameric ligand-gated ion channels mediating fast cholinergic transmission.[1−3]

(+)-Dihydro-β-erythroidine [DHβE (**1**), Figure 1B] was previously used in the treatment of Parkinson's disease to relieve tetanus and spastic disorders. As one of the most potent members of the Erythrina alkaloid family, DHβE (**1**) has become a key pharmacological tool in the nAChR field as it displays pronounced subtype-selectivity for β2-subunit-containing nAChRs (*e.g.*, α4β2) over β4-containing (*e.g.*, α3β4) and other nAChR subtypes (*e.g.*, α7).[4,5,6a−c] **1** and the other Erythrina alkaloids act as competitive antagonists at the nAChR, and their binding modes to the receptors have been elucidated by a co-crystal structure of **1** and the ACh-binding protein (AChBP), a mollusk protein homologous to the extracellular domain of the nAChR.[6c]

The Erythrina alkaloids all possess a structurally rigid spiroamine scaffold, where the A-, B-, and C-rings are generally decorated in an oxidatively similar manner—Erythrivarine B (**2**) represents an exception to this. Most subcategories of Erythrina alkaloids distinguish themselves from each other in the nature of their D-ring, where lactones, aryls, and heteroaryls comprise the majority of the reported moieties (Figure 1B).[7−9]

The structural complexity and pharmacological profile of Erythrina alkaloids have made them popular targets for total synthesis, resulting in several notable approaches.[10a−m,11] However, only a few of these efforts have produced optically pure natural products.[7,10a,11]

Moreover, the oxidative diversity found in the D-ring of Erythrinanes has also made a unifying synthetic approach to various subcategories very challenging. In the present work,[12,14,16] we expand upon our previously developed synthetic route to the Erythrina alkaloids and demonstrate that our divergent synthetic strategy allows access to the lactonic and aromatic Erythrina natural products for the first time.[10k,l]

We also characterize the binding and functional properties of this series of Erythrina alkaloids at nAChRs. In a previous investigation, we found that the AB-ring system of DHβE (**1**) is bound deep in the nAChR-binding pocket with the lactonic D-ring protruding from it (Figure 2A).[6c] In the present study, we take advantage of our recently disclosed total synthesis of **1** to access a library of ligands.[10k,l] These ligands maintain the structural features of **1** embedded in the binding pocket, while allowing for easy manipulation of the part protruding from the binding pocket (Figure 2B). The MeO-appendage on the A-ring established an interaction with a water molecule in the binding pocket, and we also wanted to probe the importance of this interaction by varying the stereochemical orientation at

Received: March 25, 2021
Published: June 1, 2021

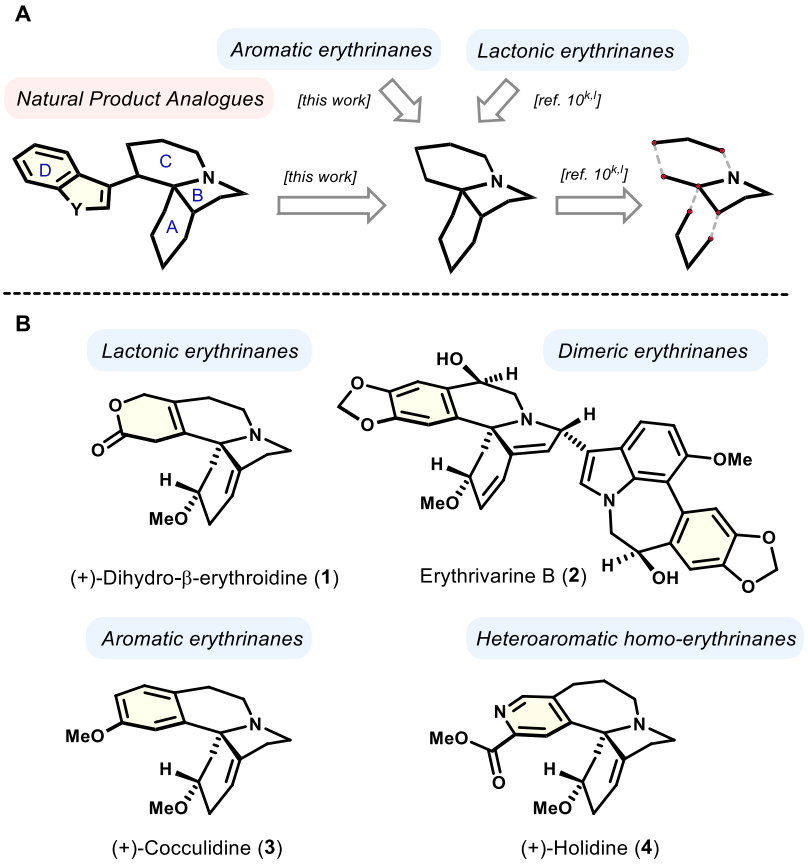


Figure 1. (A) General overview of a divergent approach to various Erythrina alkaloid subcategories and (B) representative members of the alkaloid family.

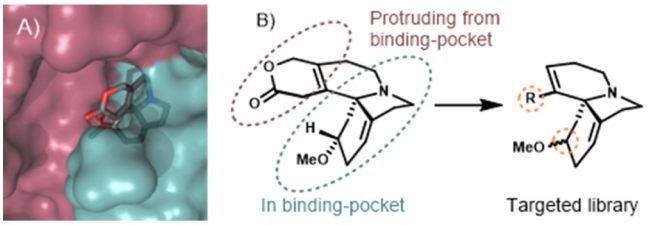


Figure 2. (A) DHβE (**1**) bound in the nAChR model.[6b] (B) Outline of the contributions of different parts of DHβE (**1**) in its nAChR binding and the targeted library, wherein R and the stereochemistry at C3 varied.

this carbon—given that our synthetic route provides access to both diastereisomers of the DHβE-scaffold.

RESULTS AND DISCUSSION

Our synthesis commenced with revisiting our total synthesis of **1** (see Scheme 1). Asymmetric allylic allylation using Trost ligand **7** delivered allyl prolinone **8**.[12a,b] The terminal double bond was then oxidatively cleaved, and the product isolated as the corresponding dimethyl acetal **9**.[13,14a,b] Methenylation of the ketone moiety proved problematic, and all attempts at using titanium carbenoids[15a–g] or addition of organometallic sources of methylene[16a,b] failed. Wittig procedures using potassium *tert*-butoxide furnished the desired olefin **10**; however, the reaction suffered from solubility issues and the yield was consistently around 50%.[17] Both aspects were resolved by forming the ylide in a separate flask and then transferring the said ylide-solution to the neat ketone,[18] which resulted in faster reaction time and increased scalability and yield. This is a notable improvement to the previously reported total synthesis.

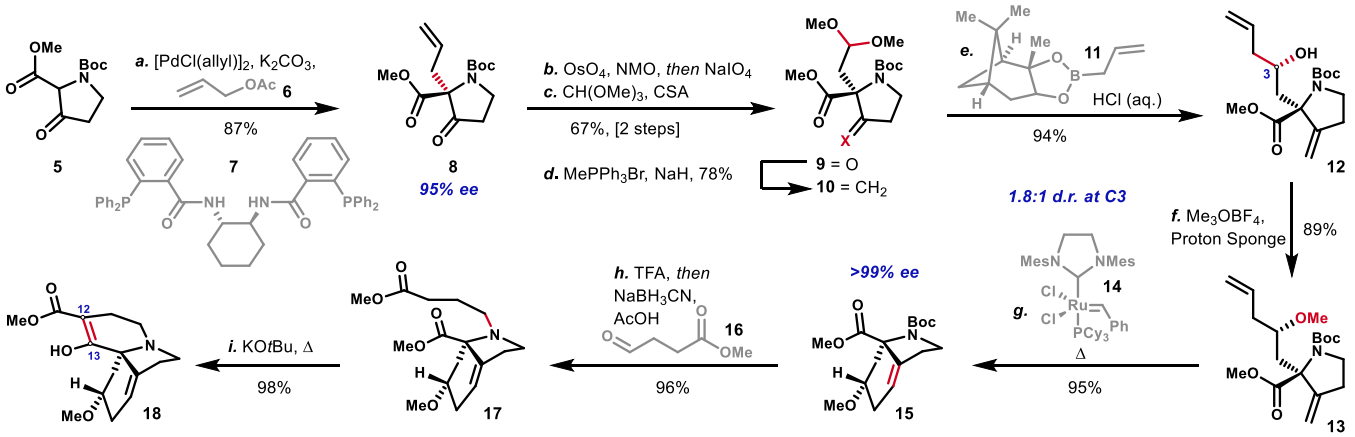
Subsequent allylation of the C3 with (+)-allylboronic acid pinanediol ester **11**[19] generated the desired diastereomer **12** in total 60% yield. Methylation and ring closing metathesis[20a,b] furnished the bicyclic ester **15**, and this compound could be further enantioenriched through the means of chiral chromatography. The final carbons of the C-ring were introduced through a one pot deprotection—reductive amination.[20,28–31] The tricyclic ring system could then be assembled by refluxing *bis*-ester **17** with potassium *tert*-butoxide, furnishing **18** in 98% yield. Further functional group manipulation produced the silyl-protected triflate **19** in 73% (see Scheme 2). Installation of the final two carbons of **1** proved problematic as the triflate **19** rapidly decomposed under basic coupling conditions.[21a–d] Eventually, we found that a base-free decarboxylative α-Cyanation formed the desired carbon—carbon bond, and the process was ultimately telescoped to **1**, using HCl.[22]

Total Synthesis of Aromatic Erythrinanes (+)-Cocculine and (+)-Cocculidine. We then turned our attention to the aromatic Erythrinanes looking to utilize intermediate **18** to access representative members from this structural class. The required carbons of the D-ring could be introduced *via* a Michael addition with methyl-vinyl-ketone **22** in quantitative yield (see Scheme 2).

Decarboxylation—condensation of **23** with potassium hydroxide in two iterations delivered enone **24**/**24′** as a 1.8:1 mixture of C-12 epimers. Enone **24** could later be

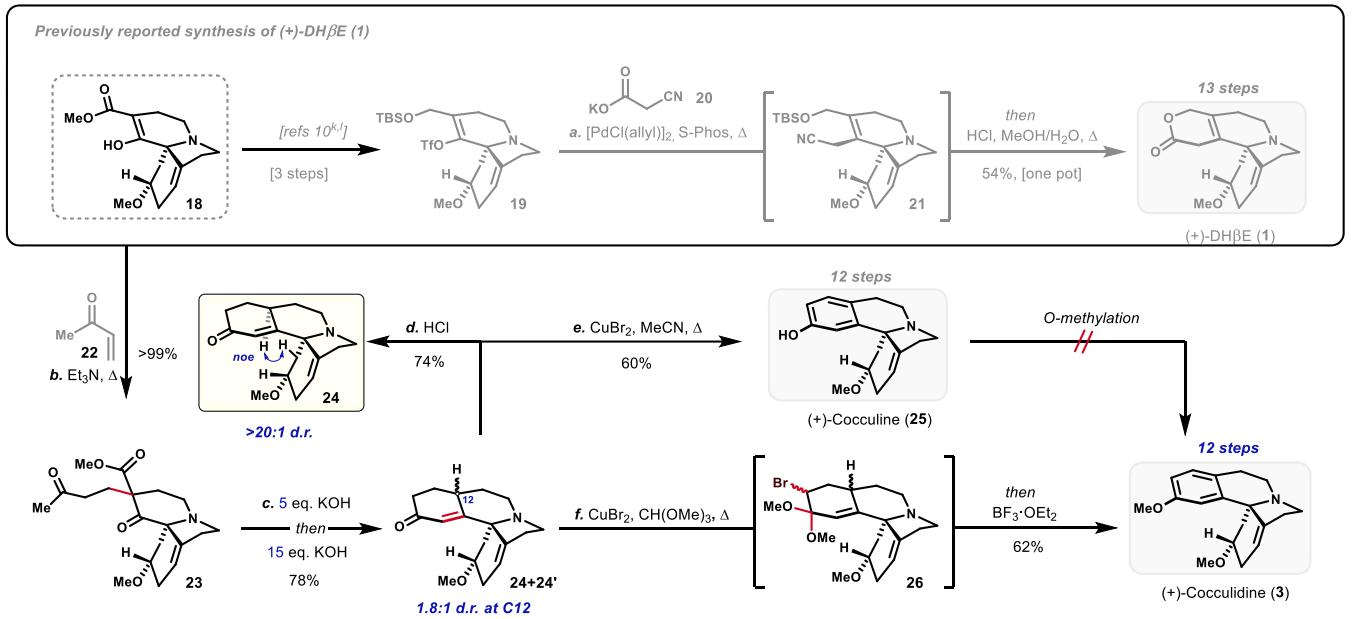
https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248–8262

Scheme 1. Previous Synthesis of Intermediate 18[10k,l,a]



[a]Reagents and conditions: (a) **6**, **7**, $[PdCl(allyl)]_2$, K_2CO_3, DMF, −40−0 °C. (b) OsO_4, NMO, THF:H_2O, rt, 12 h, then $NaIO_4$, DCM, rt, 4 h. (c) $CH(OMe)_3$, CSA, toluene, rt, 2 h. (d) $MePPh_3Br$, NaH, toluene, 90 °C, 10 h, then **9**. (e) **11**, HCl, DCM: H_2O, rt, 8 h, d.r. 1.8:1. (f) Me_3OBF_4, proton-sponge, DCM, rt, 3 h. (g) **14**, toluene, 80 °C. (h) TFA:DCM, rt, 30 min, then **16**, AcOH, $NaBH_3CN$, THF:MeOH, rt. (i) KOtBu, toluene, 95 °C, 1 h.

Scheme 2. Total Synthesis of (+)-DHβE (1), (+)-Cocculine (25), and (+)-Cocculidine (3)[a]



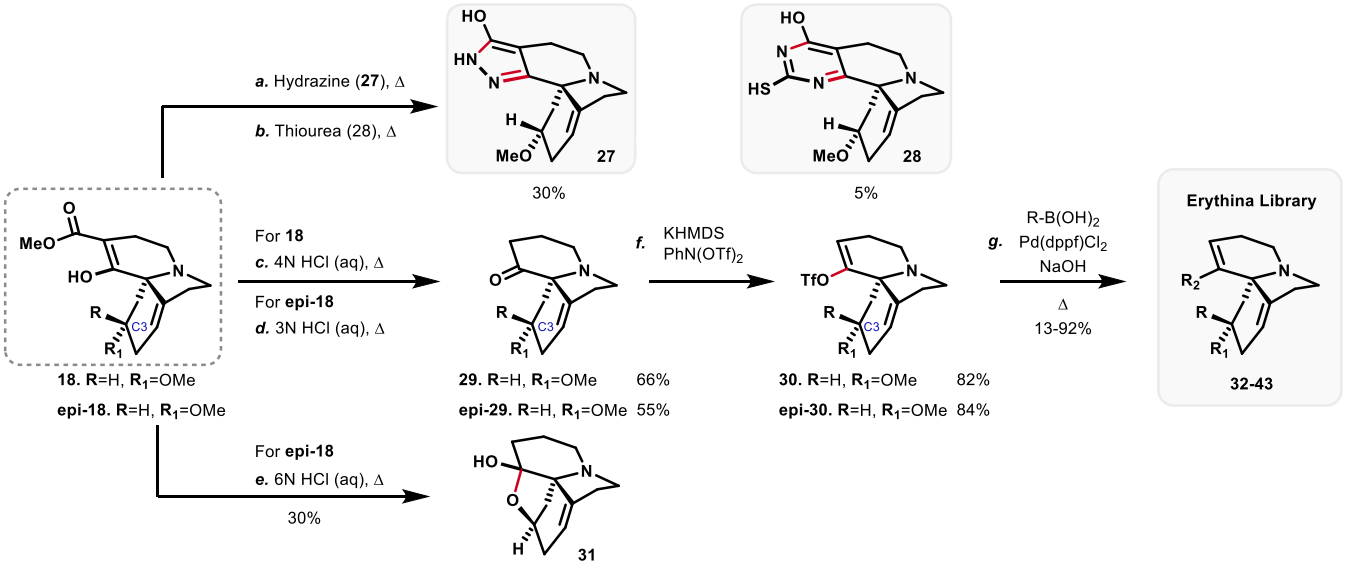
[a]Reagents and conditions: (a) **20** (3 equiv), $[PdCl(allyl)]_2$ (0.2 equiv), S-Phos (0.4 equiv), mesitylene, 110 °C, 30 min, then HCl (aq), MeOH, 85 °C, 30 min, 54%. (b) Et_3N (5 equiv), **22** (5 equiv), MeOH, 70 °C, 14 h, >99%. (c) KOH (5 equiv), water, 105 °C, 2 h, then KOH (15 equiv), 105 °C, 2h, 78%. (d) HCl (6.3 equiv), $CHCl_3$, room temperature, 24 h, 74%. (e) $CuBr_2$ (4 equiv), MeCN, 40 °C, 5 h, 60%. (f) $CuBr_2$ (2 equiv), $CH(OMe)_3$ (15 equiv), methanol, 80 °C, 14 h, then $BF_3{\cdot}OEt_2$ (2 equiv), DCM, 0 °C-rt, 2 h, 62%. S-Phos = 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl. DCM = dichloromethane.

obtained as a single epimer in 56% yield following treatment with HCl. The final oxidation of the D-ring proved difficult (see the Supporting Information) but was ultimately accomplished using stoichiometric amounts of $CuBr_2$,[23] delivering the phenolic natural product (+)-Cocculine **25** in 60% yield. Standard O-methylation procedures (methyl iodide or diazomethane) did not produce (+) Cocculidine (**3**), but upon treatment of enone **24** with $CuBr_2$ in the presence of trimethyl orthoformate, the bromo-ketal **26** (tentatively assigned) was formed as the major component. Subsequent Lewis acid-mediated elimination delivered (+)-Cocculidine (**3**) in good 62% yield from **24** (see the Supporting Information for a complete list of attempted oxidations). This approach represents the first synthesis of (+)-Cocculine (**25**) as well as the shortest and first asymmetric synthesis of (+)-Cocculidine (**3**), both in 12 steps, respectively.

Synthesis of Erythrina Library. Using key intermediate **18** as the starting point, we set out to access a library of Erythrina alkaloid analogues (see Scheme 3). To be able to investigate the significance of the stereochemistry of the C3 methyl ether, both epimers **18** and **epi-18** were decarboxylated to ketones **29** and **epi-29** using 4 and 3 molar aqueous HCl, respectively. Interestingly, when exposed to 4−6N HCl, the C3-epimer underwent O-demethylation and subsequent lactonization to form the tetracyclic lactone **31**. Triflation of **29** and **epi-29** with KHMDS and bis-triflimide[24] generated the

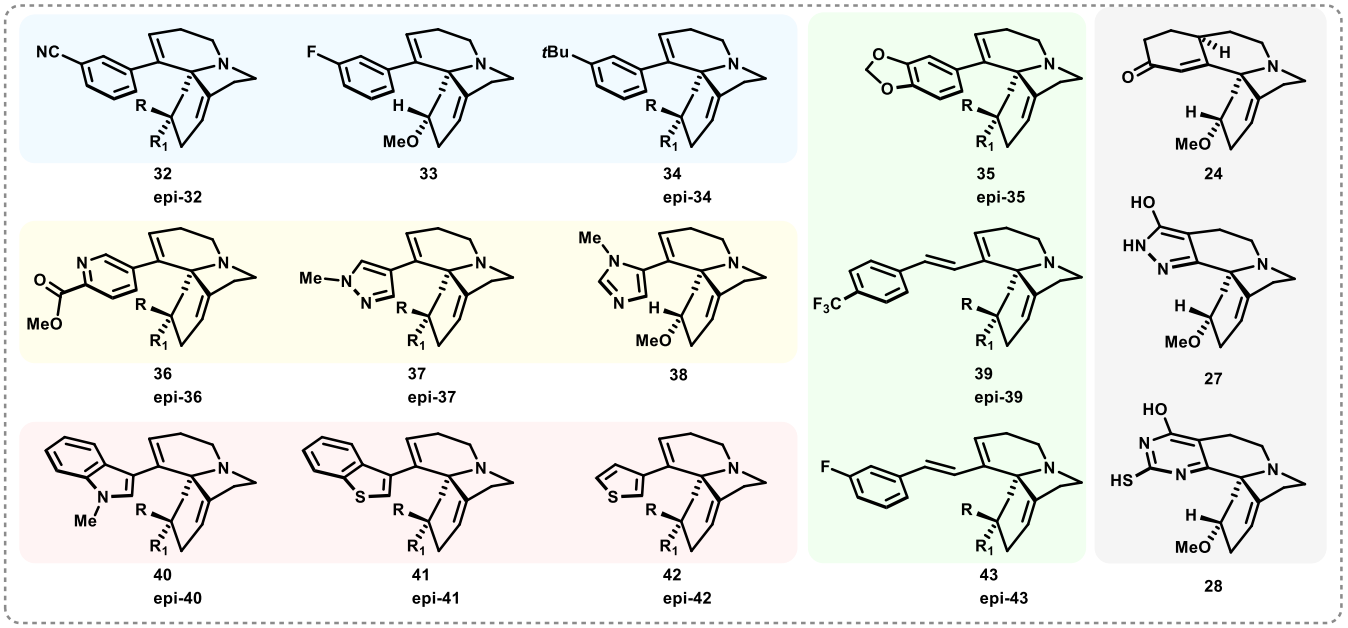
https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

Scheme 3. Synthesis of Erythrina Analogues[a]



[a]Reagents and conditions: (a) Hydrazine−hydrate (10 equiv), 1,4-dioxane, 100 °C, 72 h, 30%. (b) Thiourea (2.6 equiv), KOtBu (2.6 equiv), MeOH, 90 °C, 12 h, 5%. (c) HCl (4N), 110 °C, 3 h, 68%. (d) HCl (3N), 110 °C, 3 h, 56%. (e) HCl (6M), 115 °C, 16 h, 30%. (f) KHMDS (2 equiv), $PhN(OTf)_2$ (1.8 equiv), THF, −78−0 °C, 3.5 h, 68%, resp. 56%. (g) $Pd(dppf)Cl_2$ (0.1 equiv), $R-B(OH)_2$ (1.7 equiv), NaOH (1.8 equiv), DME, 85 °C, 75 min. DME = dimethoxyethane. KHMDS = potassium *bis*(trimethylsilyl)amide. THF = tetrahydrofuran.

Scheme 4. Structures of Ligands in the Erythrina Library Accessed in Scheme 3



corresponding triflates **30** and **epi-30**, which set the stage for the assembly of the final carbon−carbon bond—consequently introducing the D-ring as a non-fused, appended ring system. Following a standard Suzuki coupling[24] of the triflates, a library containing over 22 unnatural Erythrina alkaloids bearing various functionalities in their D-ring was synthesized.

With the exception of **33** and **38**, both epimers were obtained from the analogues **32**−**43**. Furthermore, **18** was condensed with hydrazine and thiourea to give heterocyclic compounds **27** and **28**, respectively. See Scheme 4 for the structure of all the ligands in the Erythrina library.

Pharmacological Characterization of the Erythrina Alkaloids at nAChRs. The binding properties of the Erythrina alkaloids as nAChR ligands were determined at two β2-containing ($\alpha4\beta2$ and $\alpha6/\alpha3\beta2\beta3$V9′S, a surrogate for the $\alpha6\beta2\beta3$ receptor) and two β4-containing ($\alpha3\beta4$ and $\alpha4\beta4$) subtypes in a [^{3}H]epibatidine competition binding assay, as previously described (see Table 1).[25a,b] The functional properties of the compounds as nAChR antagonists were investigated at the $\alpha4\beta2$ and $\alpha3\beta4$ receptors in a Ca^{2+} imaging assay using the Ca2+ fluorophore Fluo-4.[25b] Full details on all of the synthesized compounds are given in Tables 1 and 2.

In agreement with the literature, **1** was found to exhibit pronounced β2-over-β4 selectivity at the nAChRs, both in terms of its binding affinities and functional antagonist potencies (see Table 1). In striking contrast and highly

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

Table 1. Binding Affinities of the *Erythrina* Alkaloid Analogues at hα4β2-, hα6/α3β2β3$^{V9'S}$-, rα3β4-, and rα3β4-HEK293 Cell Membranes in the [^{3}H]epibatidine Competition Binding Assay[a]

	hα4β2	hα6/α3β2β3$^{V9'S}$	rα3β4	rα4β4
	K_i [pK_i ± S.E.M.]$^{(n)}$	K_i [pK_i ± S.E.M.]$^{(n)}$	K_i [pK_i ± S.E.M.]$^{(n)}$	K_i [pK_i ± S.E.M.]$^{(n)}$
(+)-DHβE (1)	0.27 [6.57 ± 0.03]$^{(4)}$	0.60 [6.22 ± 0.10]$^{(4)}$	~50 [~4.3]$^{(3),c}$	~20 [~4.7]$^{(3),c}$
(−)-DHβE (1′)	~30 [~4.5]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
3	0.0049 [8.31 ± 0.08]$^{(3)}$	0.075 [7.13 ± 0.01]$^{(3)}$	~20 [~4.7]$^{(3),b}$	3.4 [5.47 ± 0.08]$^{(3)}$
18	0.60 [6.22 ± 0.05]$^{(4)}$	1.6 [5.80 ± 0.12]$^{(3)}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
epi-18	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
24	0.59 [6.23 ± 0.03]$^{(3)}$	4.0 [5.39 ± 0.02]$^{(3)}$	IC_{50} > 100 $^{(3),b}$	~20 [~4.7]$^{(3),c}$
25	0.0018 [8.75 ± 0.13]$^{(3)}$	0.042 [7.37 ± 0.11]$^{(3)}$	~10 [~5.0]$^{(3),c}$	1.2 [5.91 ± 0.05]$^{(3)}$
27	~10 [~5.0]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
28	~20 [~4.8]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
29	4.3 [5.37 ± 0.09]$^{(4)}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
epi-29	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
31	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
32	4.8 [5.32 ± 0.06]$^{(4)}$	4.6 [5.34 ± 0.10]$^{(3)}$	~50 [~4.3]$^{(3),c}$	~50 [~4.3]$^{(3),c}$
epi-32	~10 [~5.0]$^{(3),c}$	7.0 [5.16 ± 0.09]$^{(3)}$	~50 [~4.3]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$
33	2.0 [5.70 ± 0.04]$^{(3)}$	3.4 [5.47 ± 0.10]$^{(3)}$	~50 [~4.3]$^{(3),c}$	~20 [~4.7]$^{(3),c}$
34	3.4 [5.46 ± 0.07]$^{(4)}$	2.9 [5.54 ± 0.10]$^{(3)}$	~50 [~4.3]$^{(3),c}$	~20 [~4.7]$^{(3),c}$
epi-34	~10 [~5.0]$^{(3),c}$	~10 [~5.0]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
35	0.39 [6.40 ± 0.04]$^{(3)}$	1.9 [5.71 ± 0.13]$^{(3)}$	~50 [~4.3]$^{(3),c}$	4.9 [5.31 ± 0.07]$^{(3)}$
epi-35	4.8 [5.32 ± 0.10]$^{(3)}$	7.1 [5.15 ± 0.08]$^{(3)}$	~50 [~4.3]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$
36	1.2 [5.92 ± 0.02]$^{(3)}$	1.8 [5.75 ± 0.09]$^{(4)}$	~50 [~4.3]$^{(3),c}$	~30 [~4.5]$^{(3),c}$
epi-36	~30 [~4.5]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
37	0.22 [6.66 ± 0.03]$^{(4)}$	0.88 [6.06 ± 0.06]$^{(4)}$	~30 [~4.5]$^{(3),c}$	2.9 [5.54 ± 0.09]$^{(n)}$
epi-37	5.5 [5.26 ± 0.05]$^{(3)}$	4.2 [5.38 ± 0.07]$^{(3)}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
38	1.3 [5.90 ± 0.05]$^{(3)}$	0.96 [6.01 ± 0.11]$^{(3)}$	~50 [~4.3]$^{(3),c}$	~20 [~4.7]$^{(3),c}$
39	0.87 [6.06 ± 0.06]$^{(4)}$	0.70 [6.16 ± 0.11]$^{(3)}$	~20 [~4.7]$^{(3),c}$	~50 [~4.3]$^{(3),c}$
epi-39	0.63 [6.20 ± 0.01]$^{(3)}$	0.33 [6.48 ± 0.08]$^{(3)}$	~10 [~5.0]$^{(3),c}$	~20 [~4.7]$^{(3),c}$
40	1.2 [5.94 ± 0.08]$^{(4)}$	1.4 [5.86 ± 0.09]$^{(3)}$	~50 [~4.3]$^{(3),c}$	~10 [~5.0]$^{(3),c}$
epi-40	2.8 [5.55 ± 0.08]$^{(3)}$	5.2 [5.29 ± 0.06]$^{(3)}$	~30 [~4.5]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$
41	0.81 [6.09 ± 0.06]$^{(3)}$	1.8 [5.75 ± 0.08]$^{(3)}$	~20 [~4.7]$^{(3),c}$	~10 [~5.0]$^{(3),c}$
epi-41	6.5 [5.19 ± 0.01]$^{(3)}$	4.7 [5.33 ± 0.05]$^{(3)}$	~30 [~4.5]$^{(3),c}$	IC_{50} > 100 $^{(3),b}$
42	3.0 [5.52 ± 0.02]$^{(4)}$	1.4 [5.87 ± 0.12]$^{(2)}$	IC_{50} > 100 $^{(3),b}$	~50 [~4.3]$^{(3),c}$
epi-42	~10 [~5.0]$^{(3),c}$	6.0 [5.22 ± 0.01]$^{(3)}$	IC_{50} > 100 $^{(3),b}$	IC_{50} > 100 $^{(3),b}$
43	0.83 [6.08 ± 0.04]$^{(3)}$	1.1 [5.97 ± 0.06]$^{(3)}$	~20 [~4.7]$^{(3),c}$	~20 [~4.7]$^{(3),c}$
epi-43	1.0 [5.98 ± 0.03]$^{(3)}$	0.23 [6.63 ± 0.10]$^{(3)}$	~10 [~5.0]$^{(3),c}$	~50 [~4.3]$^{(3),c}$

[a]The binding affinities of the compounds are given as K_i values in μM [with pK_i ± standard error of mean (S.E.M.) in brackets] with the number of experiments (n) indicated in superscript. [b]At 100 μM, the compound mediated less than 50% inhibition of specific [^{3}H]epibatidine binding (*i.e.*, IC_{50} > 100 μM). [c]The concentration−inhibition relationship for the compound was not complete at 100 μM. Thus, the K_i value for the compound was calculated based on an IC_{50} value estimated by visual inspection from the data.

interestingly, however, an overall difference was observed for these properties exhibited by most of the 33 other Erythrina alkaloids. However, all active analogues analogously to **1** displayed significantly higher binding affinities to α4β2 and α6/α3β2β3V9′S than to α3β4 and α4β4, they displayed substantially lower degrees of α4β2-over-α3β4 selectivity in the Ca^{2+}/Fluo-4 assay, so much so that many of the analogues were equipotent antagonists at these two receptors. Since the binding affinities and antagonist potencies exhibited by the analogues at the α4β2 nAChR correlated fairly well (Figure S9), the observed loss of functional β2-over-β4 selectivity arises from the analogues possessing higher α3β4 activity in this Ca2+/Fluo-4 assay than that in the binding assay. Since data from radioligand-binding competition binding experiments predominantly are believed to reflect the binding affinity of the ligand to a desensitized nAChR conformation, the antagonist potency of the competitive antagonist in a functional assay mainly reflects its binding to the resting receptor conformation. The apparent discrepancy between the β2-over-β4 selectivity exhibited by the Erythrina alkaloids in the two assays could thus be speculated to be a reflection of different binding properties of the compounds to different receptor conformations, in particular in the case of α3β4 and other β4-containing nAChRs. Overall, the accessed Erythrina ligands retained the high affinity/antagonist potency of **1** at the two β2-containing nAChRs. Among the exceptions was the significant loss of activity in the heterocyclic derivatives **27**, **28**, **36**, and **42**. Strikingly, with low nanomolar K_i values at α4β2 and α6/α3β2β3V9′S, natural products (+)-Cocculine (**25**) and (+)-Cocculidine (**3**) displayed 10−100-fold higher binding affinities at the two β2-containing nAChRs than **1** itself, and these two natural products exhibited pronounced β2-over-β4 selectivity in both binding and functional assays. Finally, comparison of the binding affinities exhibited by the 10 epimeric pairs at the two β2-containing nAChRs reveals very similar K_i values for the **32**/**epi-32**, **40**/**epi-40**, and **43**/**epi-43** epimers, whereas for the seven other epimeric pairs, it is the natural (*S*)-epimer that is a somewhat better binder than the

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

Table 2. Functional Properties Displayed by the *Erythrina* Alkaloid Analogues at h$\alpha4\beta2$-HEK293 and r$\alpha3\beta4$-HEK293 Cell Lines in the Ca^{2+}/Fluo-4 Assay[a]

	h$\alpha4\beta2$	r$\alpha3\beta4$
	IC_{50} [pIC_{50} ± S.E.M.]$^{(n)}$	IC_{50} [pIC_{50} ± S.E.M.]$^{(n)}$
(+)-DHβE (**1**)	0.35 [6.45 ± 0.06]$^{(6)}$	>100 [<4.0]$^{(6),b}$
(−)-DHβE (**1′**)	∼30 [∼4.5]$^{(3),c}$	∼100 [∼4.0]$^{(3),b}$
3	0.26 [6.59 ± 0.10]$^{(4)}$	4.2 [5.37 ± 0.12]$^{(4)}$
18	4.8 [5.32 ± 0.08]$^{(3)}$	>100 [<4.0]$^{(3),b}$
epi-18	>100 [<4.0]$^{(3),b}$	>100 [<4.0]$^{(3),b}$
24	2.0 [5.69 ± 0.08]$^{(3)}$	∼100 [∼4.0]$^{(3),c}$
25	0.12 [6.92 ± 0.05]$^{(4)}$	∼20 [∼4.7]$^{(3),c}$
27	∼100 [∼4.0]$^{(3),c}$	>100 [<4.0]$^{(3),b}$
28	∼20 [∼4.7]$^{(3),c}$	>100 [<4.0]$^{(3),b}$
29	>100 [<4.0]$^{(3),b}$	>100 [<4.0]$^{(3),b}$
epi-29	>100 [<4.0]$^{(3),b}$	>100 [<4.0]$^{(3),b}$
31	>100 [<4.0]$^{(3),b}$	>100 [<4.0]$^{(3),b}$
32	7.4 [5.13 ± 0.07]$^{(3)}$	2.6 [5.59 ± 0.11]$^{(3)}$
epi-32	2.1 [5.68 ± 0.07]$^{(3)}$	5.0 [5.30 ± 0.07]$^{(3)}$
33	0.49 [6.31 ± 0.08]$^{(4)}$	1.6 [5.79 ± 0.14]$^{(4)}$
34	1.5 [5.81 ± 0.10]$^{(3)}$	2.6 [5.89 ± 0.08]$^{(3)}$
epi-34	0.99 [6.00 ± 0.07]$^{(3)}$	4.2 [5.38 ± 0.07]$^{(3)}$
35	0.33 [6.49 ± 0.02]$^{(3)}$	0.79 [6.10 ± 0.05]$^{(3)}$
epi-35	0.33 [6.49 ± 0.12]$^{(3)}$	1.2 [5.91 ± 0.06]$^{(3)}$
36	∼20 [∼4.7]$^{(3),c}$	∼30 [∼4.5]$^{(3),c}$
epi-36	∼20 [∼4.7]$^{(3),c}$	∼50 [∼4.3]$^{(3),c}$
37	1.8 [5.75 ± 0.11]$^{(3)}$	11 [4.96 ± 0.06]$^{(3)}$
epi-37	5.6 [5.25 ± 0.13]$^{(3)}$	∼30 [∼4.5]$^{(3),b}$
38	∼20 [∼4.7](3),^{c}	∼100 [∼4.0](3),^{c}
39	1.3 [5.89 ± 0.11]$^{(3)}$	5.7 [5.25 ± 0.09]$^{(3)}$
epi-39	1.1 [5.94 ± 0.11]$^{(4)}$	5.6 [5.25 ± 0.06]$^{(3)}$
40	0.52 [6.29 ± 0.10]$^{(3)}$	2.9 [5.54 ± 0.03]$^{(3)}$
epi-40	0.14 [6.84 ± 0.12]$^{(3)}$	1.6 [5.80 ± 0.11]$^{(4)}$
41	0.24 [6.62 ± 0.07]$^{(3)}$	0.78 [6.11 ± 0.07]$^{(3)}$
epi-41	0.44 [6.36 ± 0.08]$^{(3)}$	1.5 [5.82 ± 0.04]$^{(3)}$
42	1.2 [5.91 ± 0.09]$^{(4)}$	2.6 [5.59 ± 0.09]$^{(3)}$
epi-42	1.3 [5.89 ± 0.08]$^{(4)}$	2.5 [5.61 ± 0.10]$^{(3)}$
43	0.29 [6.54 ± 0.07]$^{(3)}$	1.5 [5.82 ± 0.06]$^{(3)}$
epi-43	0.54 [6.27 ± 0.13]$^{(3)}$	1.1 [5.96 ± 0.10]$^{(3)}$

[a]The antagonist properties were determined using (S)-nicotine ∼EC_{80} (EC_{70}–EC_{90}) as an agonist. Antagonist potencies are given as IC_{50} values in μM [with pIC_{50} ± standard error of mean (S.E.M.) in brackets] with the number of experiments (n) indicated in superscript. [b]No significant inhibition observed at 100 μM. [c]The concentration–inhibition relationship was not complete at 100 μM. The IC_{50} value was estimated from the fitted curve by visual inspection.

unnatural (*R*)-epimer. The most prominent example of the latter is the 30–100-fold higher binding affinities exhibited by **36** compared to **epi-36** at $\alpha4\beta2$ and $\alpha6/\alpha3\beta2\beta3$V9′S.

Elucidation of nAChR SAR Properties Using $\alpha4\beta2$ Homology Model. In order to aid the interpretation of the pharmacological properties exhibited by the alkaloids at the nAChRs, all analogues were docked in the homology model of the $\alpha4\beta2$ nAChR reported by Yu *et al*[6b] (see the Supporting Information for details). Docking of **1** into this homology model replicated the binding mode exhibited by the compound in the AChBP/DHβE co-structure.[6c] All synthesized ligands could be accommodated in the binding pocket, where they adopted a similar conformation to **1** (see Figure 3a): (1) a H-bond from the protonated amine to the backbone carbonyl of W156 in the α4-subunit; (2) a H-bond from the methoxy group to the water molecule; and (3) the varying substituents protruding toward the β2 subunit. In the proposed binding mode from the docking of **3** and **25**, the aromatic ring forms π-stacking interactions to W57 of the β2 (t-shaped) and possibly also to Y197 of the α4 subunit (parallel) (see Figure 3b). This interaction is stronger compared to the van der Waals interactions of **1** and could explain the very high binding affinity of these two compounds compared to **1**. In contrast, the two heterocyclic derivatives **27** and **28** have a hydroxyl group protruding into this area, and the unfavorable interactions arising from this could be the root of the significantly reduced binding affinity exhibited by these analogues (see Figure 3c).

A total of 10 epimeric ligand pairs were accessed. In general, the inversion of the C3-stereocenter does not have a significant impact to the affinity of the ligands for $\alpha4\beta2$—with the notable exception of **36**/**epi-36**, where the epimer displayed a substantially lower binding affinity. When docked into the $\alpha4\beta2$ model, most epimers exhibited the highest scoring binding pose, where the fused ring system is overlapping with **1** and the methoxy group is adopting a pseudo-axial position with no H-bond to the water molecule (Figure 3e). This is independent of including the water molecule found in the X-ray structure in the docking. Thus, the importance of the interaction with the water molecule found in the X-ray structure for binding of Erythrina alkaloids may be overestimated. As the size of the substituent in the Erythrina alkaloid is increased (**39** and **43**), binding modes wherein the AB-ring systems rotate in the binding pocket become predominant for the epimeric derivatives, as illustrated with **epi-43** in Figure 3f. This suggests that the styryl derivatives **epi-39** and **epi-43** may have a different binding mode than the rest of the epimer analogues.

CONCLUSIONS

A library of Erythrina alkaloid derivatives were accessed in a concise and convergent manner from a common intermediate, giving access to ligands with a complex molecular architecture. The enantioselective total synthesis of two Erythrina alkaloids was completed. Subsequently, the pharmacological properties of the synthesized analogues at nAChRs were determined in binding and functional assays. The natural products (+)-Cocculidine (**3**) and (+)-Cocculine (**25**) exhibited remarkably higher affinities at $\alpha4\beta2$ and h$\alpha6/\alpha3\beta2\beta3$V9′S and higher β2-over-β4 selectivity than (+)-DHβE (**1**) in the binding assay and thus constitute promising leads for future nAChR ligand development. Also, notably was the loss of the inherent $\alpha4\beta2$-over-$\alpha3\beta4$ selectivity observed for most of the synthetic analogues in the functional assay as this indicates that the nAChR selectivity profile of the Erythrina alkaloids can be tweaked by structural modifications to its scaffold. Finally, a set of epimeric ligands were used to investigate a specific interaction with a water molecule in the receptor, which showed that the binding pocket can accommodate both epimers with comparable affinity. In conclusion, the Erythrina alkaloids constitute a promising scaffold for ligand development in the nAChR and potentially other fields, and the divergent synthetic strategy presented in this work will enable the exploration of this potential by allowing access to diverse series of derivatives.

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248–8262

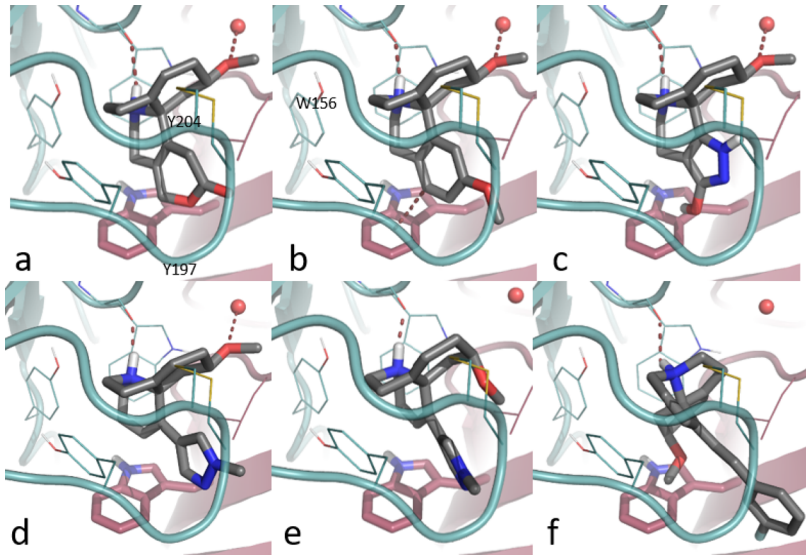


Figure 3. Docking poses exhibited by (a) **1**, (b) **3**, (c) **27**, (d) **37**, (e) **epi-37**, and (f) **epi-43** in the $\alpha4\beta2$ nAChR homology model.[6b]

EXPERIMENTAL SECTION

General Information. Unless otherwise stated, all reactions were performed in an oven-dried or flame-dried glassware under an atmosphere of dry nitrogen or argon. Dry tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), toluene, hexane, acetonitrile, and diethyl ether were used. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) on Merck TLC-Silica gel 60 F_{254} Aluminum sheets (5 × 7.5 cm) and visualized by UV irradiation and staining with the potassium permanganate developing agent. LC−MS data were acquired using a waters acquity UPLC−MS consisting of a waters acquity system including column manager, binary solvent manager, sample organizer, Photodiode-Array Detection (PAD) detector (operating at 254 nm), Evaporative light scattering (ELS) detector, and Triple Quad Mass Spectrometry (TQ−MS) equipped with atmospheric pressure photoionization-source operating in the positive ion mode. LC conditions were as follows: the column was Acquity UPLC BEH C18 1.7 μm; 2.1 × 50 mm operating at 60 °C with 1.2 mL/min binary gradient consisting of H_2O + 0.05% trifluoroacetic acid (TFA) (A) and MeCN + 5% H_2O + 0.05% TFA (B). Gradient: 0.00 min: 10% B; 1.00 min: 100% B; 1.01 min: 10% B; and 1.15 min: 10% B. The retention times provided in the Experimental Section shall be compared to the total run time of 1.20 min. Yields refer to chromatographically and spectroscopically (^{1}H NMR and ^{13}C NMR) homogeneous material, unless otherwise stated. Structural assignments were made with additional information from gradient correlation spectroscopy (gCOSY), gradient hetero-nuclear single quantum coherence (gHSQC), and gradient hetero-nuclear multiple bond correlation (gHMBC) experiments. Volatile solvents were removed under reduced pressure using a rotary evaporator. Flash column chromatography was performed using RediSep silica gel (60 Å, 230−400 mesh, 35−70 μm). Ethyl acetate and heptane were purchased from Fisher Chemical and used for chromatography without further purification. NMR data were collected with a Bruker 600-AVANCE-III spectrometer equipped with a 5 mm TCI cryoprobe operating at 600 MHz for ^{1}H and 151 MHz for ^{13}C. Chemical shifts are reported in parts per million with respect to the residual solvent signal $CDCl_3$ (^{1}H NMR: δ = 7.26; ^{13}C NMR: δ = 77.16) or D_2O (^{1}H NMR: δ = 4.79) or CD_3OD (^{1}H NMR: δ = 3.31; ^{13}C NMR: δ = 49.00) or C_6D_6 (^{1}H NMR: δ = 7.16; ^{13}C NMR: δ = 128.06), as well as using tetramethylsilane as an internal reference. Peak multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublets, td = triplet of doublets, qd = quartet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, ddt = doublet of doublet of triplets, ddq = doublet of doublet of quartets, dddd = doublet of doublet of doublet of doublets, m = multiplet, and app = apparent. Melting points were determined using a Büchi Melting Point B-540 apparatus. IR spectra were recorded on a Bruker Platinum ATR, Tensor 27 spectrometer. HRMS data were acquired with a Bruker Daltonic MicroTOF with internal calibration using electrospray ionization (ESI) in the positive mode. Optical rotations were measured on an Anton Paar MCP 300 polarimeter. Preparative supercritical fluid chromatography (SFC) was performed on a Berger Multigram II operating at 50 mL/min at 35 °C and 100 bar backpressure using stacked injections. The column was a Diacel IA CHIRALPAK (25 cm, 5μ) (250 × 20 mm). The eluent was CO_2 (95%) and ethanol + 0.1% diethylamine in ethanol (5%). UV detection was performed at 230 nM. Enantiomeric excess (*ee*) was determined on an Aurora Fusion A5/Agilent SFC system operating at 4 mL/min at 40 °C and 150 bar backpressure. The column was an AD (3× AD 3 columns in serial for compound **6**) 3μ, 15cm (150 × 4.6 mm). The eluent was CO_2 (95%) and 2-propanol (for compound **7**) or ethanol (for compounds **6** and **1**) + 0.1% diethylamine in ethanol (10%). For the synthesis and characterization of compounds **5**−**21**, and **1**, see ref 10k.

*(2S,13bS)-2,12-Dimethoxy-2,3,5,6,8,9-hexahydro-1H-indolo-[7a,1-a]isoquinoline (+)-Cocculidine (**3**).* An oven-dried 5 mL microwave vial equipped with a stir bar was charged with enone **24′** + **24** (20.0 mg, 0.073 mmol) and methanol (1.5 mL). Copper (II) bromide (33.0 mg, 0.15 mmol) and trimethylorthoformate (0.12 mL, 1.10 mmol) were added at room temperature. The vial was capped, and the reaction mixture was heated to 80 °C (using an oil bath) and stirred for 14 h. At this point, the starting material was completely consumed and the intermediate bromo-ketal is primarily formed, as indicated by TLC and LC−MS (see $[M + 2]^+$ in Figure S1). The reaction mixture was concentrated *in vacuo* and subsequently diluted with DCM (1.5 mL). Boron trifluoride diethyl etherate (18.0 μL, 0.15 mmol) was added dropwise at 0 °C, and the reaction mixture was slowly warmed to room temperature and stirred for 2 h. LC−MS at this point shows complete conversion of the intermediate to the desired mass (see $[M + H]^+$ in Figure S2). Aqueous HCl (91.0 μL, 0.37 mmol, 4 M) was added dropwise, and the resulting solution was stirred for 30 min at room temperature. The mixture was poured into a separation funnel charged with saturated aqueous $NaHCO_3$ (50 mL) and EtOAc (100 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (50 mL × 2), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N):heptane = 0:1 to 8:2], affording (+)-Cocculidine (**3**) (13.0 mg,

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

0.046 mmol, 62%) as a white solid. **^{1}H NMR** (600 MHz, C_6D_6): δ 6.97 (d, J = 2.8 Hz, 1H), 6.90 (d, J = 8.3 Hz, 1H), 6.67 (dd, J = 8.3, 2.7 Hz, 1H), 5.41−5.37 (m, 1H), 3.91 (dddd, J = 12.5, 8.7, 6.5, 4.1 Hz, 1H), 3.44 (ddd, J = 14.2, 10.8, 7.0 Hz, 1H), 3.38 (s, 3H), 3.01 (s, 3H), 2.90 (ddd, J = 14.2, 7.7, 2.1 Hz, 1H), 2.83 (td, J = 8.8, 3.2 Hz, 1H), 2.76 (ddd, J = 17.9, 10.8, 7.7 Hz, 1H), 2.63−2.56 (m, 2H), 2.52 (dd, J = 11.3, 4.0 Hz, 1H), 2.34−2.22 (m, 2H), 2.19−2.07 (m, 2H), 1.95 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, C_6D_6): δ 157.9, 142.0, 140.6, 130.6, 126.0, 117.8, 114.5, 112.0, 74.1, 64.8, 55.7, 54.8, 47.5, 42.2, 41.4, 32.6, 27.7, 21.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{18}H_{24}NO_2$, 286.1802; Found, 286.1803. **Rf** = 0.18 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +255.9° (c 1.0, MeOH). >99% *ee*. [Reference 1−$[\alpha]_D^{25}$ = +260° (c 1.0, MeOH).] **mp** = 80−82 °C. [Reference 26−m.p. 86−87]. The ^{1}H and ^{13}C NMR spectra are in congruent with previously reported spectra.[10b]

The olifination reaction leading to **10** was optimized in the following way.

*1-(Tert-butyl) 2-Methyl (S)-2-(2,2-dimethoxyethyl)-3-methylene-pyrrolidine-1,2-dicarboxylate (**10**).* An oven-dried 250 mL round-bottom flask equipped with a stir bar was charged with sodium hydride (3.62 g, 91 mmol 60% w/w), methyltriphenylphosphonium bromide (32.3 g, 90 mmol), and toluene (160 mL). The flask was equipped with a reflux condenser, and the reaction mixture was heated to 90 °C (using an oil bath) and stirred for 12 h. The reaction mixture was allowed to reach room temperature, allowing the solids to settle. The yellow supernatant was added to a toluene solution (75 mL) of acetal **9** (2.75 g, 8.3 mmol) in an oven-dried 500 mL round-bottom flask, and the resulting solution was stirred at room temperature for 12 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was poured into a separation funnel charged with water (200 mL) and EtOAc (300 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (150 mL × 3), and the combined organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was purified by column chromatography (EtOAc/heptane = 0:1 to 7:3), affording olefin **10** (2.11 g, 6.42 mmol, 78%) as a pale yellow oil. For analytical data of olein **10**, see ref 10k.

*Methyl (10S,11aS)-1-hydroxy-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole-2-carboxylate (**18**).* The title compound was synthesized, as previously described.[10k]

*Methyl (10R,11aS)-1-hydroxy-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole-2-carboxylate (**epi-18**).* The title compound was synthesized, as previously described.[10k]

The enol-esters **18** and **epi-18** were usually carried on without any purification but were on occasion purified using column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2].

*Methyl (2R,10S,11aS)-10-methoxy-1-oxo-2-(3-oxobutyl)-1,2,3,4,7,9,10,11-octahydro-6H-pyrido[2,1-i]indole-2-carboxylate (**23**).* An oven-dried 25 mL microwave vial equipped with a stir bar was charged with enol-ester **18** (100 mg, 0.36 mmol) and methanol (7 mL). Et_3N (0.25 mL, 1.79 mmol) and methyl vinyl ketone (0.15 mL, 1.79 mmol) were added at room temperature. The vial was capped, and the reaction mixture was heated to 70 °C (using an oil bath) and stirred for 14 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was cooled to room temperature and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording dione **23** (125 mg, 0.36 mmol, >99%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.64−5.60 (m, 1H), 3.68 (s, 3H), 3.54−3.44 (m, 2H), 3.29 (s, 3H), 3.09 (td, J = 9.3, 5.7 Hz, 1H), 3.05−2.98 (m, 2H), 2.87 (ddd, J = 14.3, 9.7, 6.0 Hz, 1H), 2.72−2.60 (m, 2H), 2.41−2.28 (m, 4H), 2.25−2.21 (m, 2H), 2.13 (s, 3H), 1.97−1.89 (m, 1H), 1.81 (dt, J = 14.3, 4.6 Hz, 1H), 1.45 (t, J = 12.2 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 211.0, 207.4, 172.2, 138.6, 120.8, 73.4, 71.9, 58.7, 56.3, 52.5, 48.9, 40.3, 39.2, 36.8, 31.5, 30.3, 30.1, 29.0, 28.1. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{19}H_{28}NO_5$, 350.1962; Found, 350.1965. **Rf** = 0.72 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +16.3° (c 1.0, $CHCl_3$). >99% *ee*. The stereochemistry could not be established through 2D NMR, and the structure is therefore tentatively assigned. The ^{1}H NMR shows a presence (less than 5%) of the other epimer at C-12. The peaks of the major epimer are reported.

(2S,9aR,13bS)-2-Methoxy-2,3,5,6,9,9a,10,11-octahydro-1H-indolo[7a,1-a]isoquinolin-12(8H)-one (24′ + 24). An oven-dried 25 mL microwave vial equipped with a stir bar was charged with dione **23** (125 mg, 0.36 mmol) and water (7 mL). Potassium hydroxide (100 mg, 1.79 mmol) in water (2.3 mL) was added at room temperature. The vial was capped, and the reaction mixture was heated to 105 °C (using an oil bath) and stirred for 2 h. At this point, the starting material was completely consumed, as indicated by TLC. Potassium hydroxide (300 mg, 5.37 mmol) in water (2.3 mL) was added. The reaction mixture was heated to 105 °C (using an oil bath) and stirred for another 3 h. At this point, the intermediate product was completely consumed, as indicated by TLC. The mixture was cooled to room temperature and subsequently poured into a separation funnel charged with saturated aqueous NH_4Cl (50 mL), brine (50 mL), and EtOAc (100 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (100 mL × 2), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording enone **24′** + **24** as an inseparable 1:2 diastereomeric mixture (76.0 mg, 0.28 mmol, 78% total yield) as a colorless oil. The crude diastereomeric mixture was proceeded with the reactions below.

*(2S,9aS,13bS)-2-Methoxy-2,3,5,6,9,9a,10,11-octahydro-1H-indolo[7a,1-a]isoquinolin-12(8H)-one (**24**).* An oven-dried 5 mL microwave vial equipped with a stir bar was charged with enone **24′** + **24** (20.0 mg, 0.073 mmol) and chloroform (1.5 mL). HCl (0.37 mL, 0.46 mmol, 1.25 M) in water methanol was added at room temperature. The vial was capped, and the reaction mixture was stirred for 24 h at room temperature. At this point, only one compound is visible, as indicated by crude ^{1}H NMR. The mixture was poured into a separation funnel charged with saturated aqueous $NaHCO_3$ (50 mL), brine (50 mL), and EtOAc (100 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (100 mL × 2), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording enone **24** in over 20:1 d.r. (14.8 mg, 0.054 mmol, 74%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.86−5.84 (m, 1H), 5.60−5.56 (m, 1H), 3.36−3.28 (m, 5H), 3.00 (dd, J = 14.5, 4.3 Hz, 1H), 2.98−2.91 (m, 1H), 2.89−2.85 (m, 1H), 2.77−2.71 (m, 1H), 2.57−2.50 (m, 2H), 2.45−2.29 (m, 4H), 2.18−2.12 (m, 1H), 1.99−1.88 (m, 2H), 1.72−1.63 (m, 2H), 1.46 (t, J = 12.0 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 199.6, 164.9, 139.3, 127.2, 119.8, 73.8, 67.1, 56.1, 46.7, 43.1, 37.6, 36.5, 32.9, 31.8, 29.5, 28.7, 26.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{17}H_{24}NO_2$, 274.1802; Found, 274.1802. **Rf** = 0.26 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +85.6° (c 0.5, $CHCl_3$). >99% *ee*.

(2*S*,13b*S*)-2-Methoxy-2,3,5,6,8,9-Hexahydro-1*H*-indolo-[7*a*,1-*a*]isoquinolin-12-ol (+)-Cocculine (25). An oven-dried 5 mL microwave vial equipped with a stir bar was charged with enone **24** + **24′** (20.0 mg, 0.073 mmol) and acetonitrile (1.5 mL). Copper(II) bromide (65.0 mg, 0.29 mmol) was added at room temperature. The vial was capped, and the reaction mixture was heated to 40 °C (using an oil bath) and stirred for 5 h. At this point, the starting material was completely consumed, as indicated by TLC and LC−MS [see $[M + H]^+$ in Figure S3]. The reaction mixture was concentrated *in vacuo* directly purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording (+)-Cocculine (**25**) (12.0 mg, 0.044 mmol, 60%) as a white solid. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.01 (d, J = 8.3 Hz, 1H), 6.84 (dd, J = 8.3, 2.5 Hz, 1H), 6.66 (d, J = 2.6 Hz, 1H), 5.80−5.77 (m, 1H), 3.81−3.75 (m, 1H), 3.69 (ddd, J = 14.1, 10.0, 7.2 Hz, 1H), 3.43−3.37 (m, 1H), 3.30−3.23 (m, 4H), 3.00 (ddd, J = 17.7, 10.3, 6.3 Hz, 1H), 2.93−2.79 (m, 2H), 2.78−2.70 (m, 1H), 2.63−2.55 (m, 1H), 2.38−2.28 (m, 2H), 2.19−2.12 (m, 1H), 2.08−2.02 (m, 1H). **^{13}C{1H} NMR** (151 MHz, methanol-D_4): δ 155.7, 136.7, 134.8, 130.5, 121.9, 121.4, 115.5, 113.3, 73.0, 67.1, 55.2, 47.3, 40.9, 38.9,

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

31.2, 25.9, 20.6. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{17}H_{22}NO_2$, 272.1645; Found, 272.1645. **Rf** = 0.15 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +215.5° (c 1.0, MeOH). >99% *ee.* (Reference 27)—$[\alpha]_D^{25}$ = +217—218° (c 1.0, MeOH). **mp** = 207—212 °C (Reference 27)—m.p. 216—217).

*(2S,12bS)-2-Methoxy-2,3,5,6,9,11-hexahydro-1H,8H-pyrazolo-[3′,4′:3,4]pyrido[2,1-i]indol-10-ol (**27**).* An oven-dried 5 mL microwave vial equipped with a stir bar was charged with crude enol-ester **18** (22.0 mg, 0.079 mmol) and 1,4-dioxane (1 mL). Hydrazine-hydrate (64% wt, 60 μL, 0.79 mmol) was added at room temperature. The vial was capped, and the reaction mixture was heated to 100 °C (using an oil bath) and stirred for 72 h. At this point, the starting material was completely consumed, as indicated by TLC. The reaction mixture was concentrated *in vacuo* directly purified by column chromatography (MeOH/EtOAc = 0:1 to 1:1), affording pyrazole **27** (6.00 mg, 0.02 mmol, 30%) as a clear colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.57—5.54 (m, 1H), 3.75—3.69 (m, 1H), 3.31 (s, 3H), 3.24—3.20 (m, 2H), 2.94 (td, J = 9.2, 3.2 Hz, 1H), 2.75—2.65 (m, 2H), 2.63—2.58 (m, 1H), 2.47—2.39 (m, 2H), 2.28—2.22 (m, 2H), 2.09—2.03 (m, 1H), 1.53 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 160.9, 143.7, 139.1, 119.1, 97.5, 74.2, 61.9, 56.3, 46.2, 40.9, 40.2, 31.8, 27.1, 14.0. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{14}H_{20}N_3O_2^+$, 262.1550; Found, 262.1555 **Rf** = 0.28 (silica gel, 1:1 EtOAc/MeOH) $[\alpha]_D^{25}$ = +203.8° (c 0.5, $CHCl_3$).

*(2S,13bS)-12-Mercapto-2-methoxy-2,3,5,6,8,9-hexahydro-1H-pyrimido[4′,5′:3,4]pyrido[2,1-i]indol-10-ol (**28**).* An oven-dried 5 mL microwave vial equipped with a stir bar was charged with crude enol-ester **18** (60.0 mg, 0.21 mmol), thiourea (41.0 mg, 0.54 mmol), and potassium *tert*-butoxide (120 mg, 0.54 mmol). MeOH (1.6 mL) was added at room temperature. The vial was capped, and the reaction mixture was heated to 90 °C (using an oil bath) and stirred for 12 h. At this point, the starting material was almost completely consumed, as indicated by TLC. The solution was poured into a separation funnel containing brine (20 mL) and 2-methyl-THF (20 mL). The organic layer was separated, and the pH of the water phase was adjusted to 7, using 1 M HCl before extracting it with 2-methyl-THF (3 × 20 mL). The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crude was purified by column chromatography (MeOH/EtOAc = 0:1 to 1:1), affording pyrimidine **27** (3.30 mg, 0.001 mmol, 5%) as a clear colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.32 (s, 1H), 5.87—5.83 (m, 1H), 3.69—3.62 (m, 1H), 3.38 (s, 3H), 3.36—3.26 (m, 2H), 3.18 (dd, J = 14.8, 7.6 Hz, 1H), 3.05—3.00 (m, 1H), 2.82—2.71 (m, 2H), 2.66—2.58 (m, 1H), 2.56—2.49 (m, 1H), 2.46—2.35 (m, 2H), 2.29 (dd, J = 18.4, 6.6 Hz, 1H), 2.18—2.11 (m, 1H), 1.66 (t, J = 12.3 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 173.7, 161.1, 150.0, 136.7, 123.1, 110.1, 72.8, 62.6, 56.6, 46.7, 40.6, 39.3, 31.6, 27.1, 15.3. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{13}H_{20}N_3O_2S^+$, 306.1271; Found, 306.1271 **Rf** = 0.36 (silica gel, 8:2 EtOAc/MeOH) $[\alpha]_D^{25}$ = +184.8° (c 0.3, $CHCl_3$).

*(10S,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indol-1(2H)-one (**29**).* An oven-dried 100 mL flask equipped with a stir bar was charged with bis-ester **17** (920 mg, 2.96 mmol) and toluene (33 mL). Potassium *tert*-butoxide (763 mg, 6.80 mmol) was added at room temperature, the vial was equipped with a reflux condenser, and the reaction mixture was heated to 95 °C (using an oil bath) and stirred for 1 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was cooled to room temperature and was poured into a separation funnel charged with saturated aqueous NH_4Cl (50 mL), brine (50 mL), and 2-methyl-THF (100 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (100 mL × 2), and the combined organic phase was washed with brine (200 mL), dried over $MgSO_4$, and concentrated *in vacuo*. To an oven-dried 100 mL flask equipped with a stir bar were added the resulting crude oil and 4N aqueous HCl (29 mL). The flask was equipped with a reflux condenser under argon, and the reaction mixture was heated to 110 °C (using an oil bath) for 3 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was poured into a separation funnel charged with saturated aqueous $NaHCO_3$ (100 mL) and 2-methyl-THF (100 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (100 mL × 2), and the combined organic phase was washed with brine (200 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/ 10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording ketone **29** (435 mg, 1.97 mmol, 66%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.62—5.57 (m, 1H), 3.45 (ddd, J = 14.6, 13.3, 3.4 Hz, 1H), 3.32 (s, 3H), 3.31—3.26 (m, 1H), 3.03—2.94 (m, 3H), 2.80 (td, J = 13.8, 6.8 Hz, 1H), 2.67 (ddd, J = 12.0, 3.4, 0.9 Hz, 1H), 2.57 (tddd, J = 18.2, 13.5, 8.2, 4.4 Hz, 2H), 2.41 (ddt, J = 8.7, 6.0, 2.7 Hz, 2H), 2.34 (ddt, J = 14.2, 4.6, 2.0 Hz, 1H), 2.05—2.00 (m, 1H), 1.93 (ddq, J = 17.2, 9.3, 3.2 Hz, 1H), 1.54 (t, J = 12.2 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 214.6, 137.7, 119.7, 73.6, 73.6, 56.2, 47.3, 43.0, 37.2, 36.4, 31.8, 26.9, 25.9. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{13}H_{20}NO_2$, 222.1489; Found, 222.1494. **Rf** = 0.21 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +11.7° (c 1.0, $CHCl_3$). >99% *ee.*

*(10R,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indol-1(2H)-one (**epi 29**).* An oven-dried 100 mL flask equipped with a stir bar was charged with bis-ester methyl (6*R*,7a*S*)-6-methoxy-1-(4-methoxy-4-oxobutyl)-1,2,3,5,6,7-hexahydro-7a*H*-indole-7a-carboxylate (191 mg, 0.61 mmol) and toluene (7 mL). Potassium *tert*-butoxide (158 mg, 1.41 mmol) was added at room temperature, the vial was equipped with a reflux condenser, and the reaction mixture was heated to 95 °C (using an oil bath) and stirred for 1 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was cooled to room temperature and was poured into a separation funnel charged with saturated aqueous NH_4Cl (50 mL), brine (50 mL), and 2-methyl-THF (100 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (100 mL × 2), and the combined organic phase was washed with brine (200 mL), dried over $MgSO_4$, and concentrated *in vacuo*. To an oven-dried 100 mL flask equipped with a stir bar were added the resulting crude oil and 3N aqueous HCl (15 mL). The flask was equipped with a reflux condenser under argon, and the reaction mixture was heated to 110 °C (using an oil bath) for 3 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was poured into a separation funnel charged with saturated aqueous $NaHCO_3$ (50 mL) and 2-methyl-THF (50 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (50 mL × 2), and the combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording C3-epi-ketone **29** (74 mg, 0.34 mmol, 55%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.56—5.54 (m, 1H), 3.68—3.65 (m, 1H), 3.42 (td, J = 13.9, 3.5 Hz, 1H), 3.23 (s, 3H), 2.94—2.86 (m, 5H), 2.61—2.50 (m, 2H), 2.42 (dddd, J = 18.5, 10.9, 6.2, 2.2 Hz, 1H), 2.37—2.32 (m, 1H), 2.28—2.18 (m, 2H), 2.07—2.02 (m, 1H), 1.67 (dd, J = 13.4, 2.9 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 212.2, 138.7, 118.0, 73.7, 69.6, 56.6, 46.3, 43.2, 37.3, 35.7, 29.6, 28.8, 27.6. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{13}H_{20}NO_2$, 222.1489; found, 222.1489. **Rf** = 0.3 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +88.6° (c 0.9, $CHCl_3$). >99% *ee.*

*(10S,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indol-1-yl Trifluoromethanesulfonate (**30**).* An oven-dried 25 mL microwave vial equipped with a stir bar was charged with ketone **29** (100 mg, 0.46 mmol) and THF (3.8 mL). The mixture was cooled to −78 °C before KHMDS (1.80 mL, 0.90 mmol, 0.5 M) was added. The resulting mixture was left stirring at the same temperature for 1.5 h. $PhN(OTf)_2$ (300 mg, 0.84 mmol) in 1 mL THF was added dropwise before the dry ice bath was replaced with an ice bath. The mixture was allowed to warm to 0 °C and was stirred at this temperature for 2 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was quenched with ice-cold water and was subsequently poured into a separation funnel charged with saturated aqueous NH_4Cl (50 mL), brine (50 mL), and EtOAc (100 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (100 mL × 2), and the combined organic phase was washed with brine (200 mL), dried over $MgSO_4$, and

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248—8262

concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording alkenyl triflate **30** (131 mg, 0.37 mmol, 82%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.75 (dd, J = 5.2, 2.8 Hz, 1H), 5.71−5.68 (m, 1H), 3.87 (dddd, J = 12.4, 8.0, 6.7, 4.4 Hz, 1H), 3.37 (s, 3H), 3.18 (ddd, J = 14.6, 11.7, 5.7 Hz, 1H), 3.02−2.90 (m, 3H), 2.76−2.56 (m, 3H), 2.50−2.41 (m, 2H), 2.12 (dt, J = 18.7, 5.4 Hz, 1H), 1.98 (ddtd, J = 17.7, 7.6, 3.6, 2.4 Hz, 1H), 1.52 (t, J = 12.0 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 152.1, 136.1, 123.3, 118.4 (q, J = 319.7 Hz), 116.8, 73.2, 63.9, 56.4, 46.6, 41.4, 39.8, 31.7, 27.2, 21.0. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{14}H_{19}F_3NO_4S$, 354.0981; Found, 354.0980. **Rf** = 0.25 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]D^{25}$ = +58.4° (c 1.0, $CHCl_3$). >99% *ee*.

*(10R,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indol-1-yl Trifluoromethanesulfonate (**epi-30**).* An oven-dried 5 mL microwave vial equipped with a stir bar was charged with **epi-29** (33.0 mg, 0.15 mmol) and THF (1 mL). The mixture was cooled to −78 °C before KHMDS (0.60 mL, 0.30 mmol, 0.5 M) was added. The resulting mixture was left stirring at the same temperature for 1.5 h. $PhN(OTf)_2$ (80.0 mg, 0.22 mmol) in 0.5 mL THF was added dropwise before the dry ice bath was replaced with an ice bath. The mixture was allowed to warm to 0 °C and was stirred at this temperature for 2 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was quenched with ice-cold water and was subsequently poured into a separation funnel charged with saturated aqueous NH_4Cl (50 mL), brine (50 mL), and EtOAc (100 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (100 mL × 2), and the combined organic phase was washed with brine (200 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording C3-epi-alkenyl triflate **30** (44.0 mg, 0.13 mmol, 84%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.79 (dtd, J = 7.9, 2.5, 1.3 Hz, 1H), 5.76 (dd, J = 5.6, 2.5 Hz, 1H), 3.35 (s, 4H), 3.32 (tdd, J = 10.2, 5.8, 4.6 Hz, 1H), 3.14 (ddd, J = 14.5, 11.5, 5.2 Hz, 1H), 3.00 (td, J = 9.2, 5.5 Hz, 1H), 2.96−2.90 (m, 2H), 2.73 (dddd, J = 18.1, 11.5, 6.3, 2.5 Hz, 1H), 2.67−2.60 (m, 1H), 2.47−2.41 (m, 1H), 2.40−2.34 (m, 1H), 2.27 (ddd, J = 14.0, 5.8, 2.1 Hz, 1H), 2.09−2.04 (m, 1H), 2.04−1.97 (m, 1H), 1.74 (dd, J = 14.0, 10.2 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 152.2, 137.8, 122.5, 118.3 (q, J = 319.6 Hz), 115.9, 75.0, 63.7, 56.2, 46.5, 39.8, 38.1, 29.4, 27.3, 20.1. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{14}H_{19}F_3NO_4S$, 354.0981; Found, 354.0980. **Rf** = 0.25 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +46.3° (c 1.0, $CHCl_3$). >99% *ee*. Note: *It was not possible to remove all traces of solvent from this compound without significant decomposition.*

*Methyl (10R,11aS)-1-Hydroxy-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole-2-carboxylate (**31**).* To an oven-dried 25 mL microwave vial equipped with a stir bar were added **epi-18** (95.0 mg, 0.34 mmol) and 6N aqueous HCl (10 mL). The vial was capped, and the reaction mixture was submerged in an oil bath that was preheated to 115 °C (using an oil bath) and stirred for 16 h. At this point, the starting material was completely consumed, as indicated by TLC, and the mixture was poured into a separation funnel charged with saturated aqueous $NaHCO_3$ (50 mL) and 2-methyl-THF (50 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (50 mL × 2), and the combined organic phase was washed with brine (100 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting crude material was purified by column chromatography [EtOAc (w/10% MeOH and 5% Et_3N)/heptane = 0:1 to 8:2], affording lactol **31** (21.0 mg, 0.10 mmol, 30%) as a colorless oil. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 5.61−5.58 (m, 1H), 4.32 (dtd, J = 5.5, 2.6, 1.3 Hz, 1H), 3.29 (dt, J = 10.3, 7.9 Hz, 1H), 3.07−2.97 (m, 2H), 2.91 (td, J = 8.3, 1.2 Hz, 1H), 2.74 (s, 1H), 2.68 (dd, J = 10.6, 5.8 Hz, 1H), 2.65−2.56 (m, 1H), 2.54−2.48 (m, 1H), 2.44−2.30 (m, 2H), 2.08 (dtd, J = 13.4, 3.3, 1.5 Hz, 1H), 1.95 (tddd, J = 13.8, 12.2, 4.7, 3.4 Hz, 1H), 1.73 (td, J = 13.7, 4.0 Hz, 1H), 1.50 (d, J = 10.6 Hz, 1H), 1.31−1.24 (m, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 145.2, 117.1, 102.9, 72.8, 67.9, 49.2, 44.5, 36.6, 35.6, 35.1, 28.3, 16.6. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{13}H_{20}NO_2$, 208.1332; Found, 208.1338. **Rf** = 0.11 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +11.6° (c 0.5, $CHCl_3$). >99% *ee*.

General Procedure for the Suzuki Coupling. An oven-dried 5 mL microwave vial equipped with a stir bar was charged with alkenyl triflate **30** or **C3-epi-30** (20.0 mg, 0.06 mmol), $Pd(dppf)Cl_2$ DCM (4.60 mg, 0.006 mmol), and boronic acid or boronic ester (0.10 mmol, 1.7 equiv). Degassed DME (0.6 mL) and NaOH (0.05 mL, 2 M) were added, and the resulting solution was further degassed for 10 min. The vial was capped, and the reaction mixture was submerged in an oil bath that was preheated to 85 °C. The reaction mixture was stirred for 75 min. At this point, the starting material was completely consumed, as indicated by TLC. The mixture was cooled to room temperature and concentrated *in vacuo*. The resulting crude material was directly purified by column chromatography (EtOAc/MeOH = 1:0 to 4:6), affording Suzuki-adducts **32**−**43** and **epi-32**−**epi-43** as pale yellow oils. *Note: The same elution gradient was used for the purification of compounds* **32**−**43**.

*3-((10S,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido-[2,1-i]indol-1-yl)benzonitrile (**32**).* The title compound was derived from **30** and (3-cyanophenyl)boronic acid to give the desired product (15.0 mg, 0.049 mmol, 86%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.54−7.51 (m, 1H), 7.42−7.41 (m, 1H), 7.37−7.34 (m, 2H), 5.73−5.69 (m, 1H), 5.61−5.58 (m, 1H), 3.29 (ddd, J = 14.4, 11.8, 5.9 Hz, 1H), 3.09−3.03 (m, 6H), 2.76 (dddd, J = 12.5, 8.8, 6.2, 3.9 Hz, 1H), 2.64−2.53 (m, 3H), 2.41 (ddd, J = 11.6, 4.0, 0.9 Hz, 1H), 2.10−2.05 (m, 1H), 1.98 (dt, J = 19.2, 5.4 Hz, 1H), 1.82 (ddtd, J = 17.6, 8.9, 3.5, 2.0 Hz, 1H), 1.51 (t, J = 11.9 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 145.0, 141.1, 138.8, 133.5, 132.5, 130.5, 128.8, 128.1, 121.7, 119.0, 112.0, 73.7, 64.6, 56.1, 46.1, 42.8, 40.0, 31.7, 28.2, 19.6. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{23}N_2O$, 307.1805; Found, 307.1807. **Rf** = 0.32 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +97.6° (c 1.0, $CHCl_3$). >99% *ee*.

*3-((10R,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido-[2,1-i]indol-1-yl)benzonitrile (**epi-32**).* The title compound was derived from **epi-30** and (3-cyanophenyl)boronic acid to give the desired product (6.00 mg, 0.02 mmol, 30%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.51 (d, J = 7.7 Hz, 1H), 7.45 (s, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.36−7.30 (m, 1H), 5.74−5.70 (m, 1H), 5.60 (d, J = 6.8 Hz, 1H), 3.31−3.22 (m, 5H), 3.07−2.99 (m, 3H), 2.67−2.51 (m, 2H), 2.44 (dt, J = 14.8, 7.1 Hz, 1H), 2.29 (dd, J = 14.4, 5.4 Hz, 1H), 2.23−2.16 (m, 1H), 1.90 (dt, J = 18.9, 5.4 Hz, 1H), 1.50−1.43 (m, 1H), 1.09−1.01 (m, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 143.0, 141.9, 141.0, 133.9, 132.9, 130.5, 128.5, 127.3, 120.4, 119.2, 111.7, 75.2, 64.9, 56.3, 46.2, 39.9, 38.1, 29.6, 28.1, 18.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{23}N_2O$, 307.1805; Found, 307.1805. **Rf** = 0.19 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +47.2° (c 0.55, $CHCl_3$). >99% *ee*.

*(10S,11aS)-1-(3-Fluorophenyl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**33**).* The title compound was derived from **30** and (3-fluorophenyl)boronic acid to give the desired product (9.00 mg, 0.050 mmol, 53%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.20 (m, 1H), 6.95−6.89 (m, 2H), 6.84 (dt, J = 10.3, 2.1 Hz, 1H), 5.70 (dd, J = 5.2, 2.6 Hz, 1H), 5.60−5.58 (m, 1H), 3.29 (ddd, J = 14.3, 11.9, 6.0 Hz, 1H), 3.12−3.04 (m, 6H), 2.87 (tdd, J = 12.5, 6.2, 3.9 Hz, 1H), 2.68−2.52 (m, 3H), 2.42 (dd, J = 11.6, 3.9 Hz, 1H), 2.14−2.07 (m, 1H), 1.98 (dt, J = 19.1, 5.5 Hz, 1H), 1.87−1.79 (m, 1H), 1.52 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 162.4 (d, J = 245.5 Hz), 145.8 (d, J = 7.6 Hz), 141.5, 138.7, 129.3 (d, J = 8.5 Hz), 126.7, 124.9 (d, J = 2.7 Hz), 121.7, 115.9 (d, J = 21.3 Hz), 113.7 (d, J = 21.1 Hz), 73.7, 64.9, 56.0, 46.1, 42.6, 40.1, 31.7, 28.1, 19.6. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{19}H_{23}NOF$, 300.1758; Found, 300.1760. **Rf** = 0.29 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]_D^{25}$ = +88.9° (c 0.9, $CHCl_3$). >99% *ee*.

*(10S,11aS)-1-(3-(Tert-Butyl)phenyl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**34**).* The title compound was derived from **30** and (3-(*tert*-butyl)phenyl)boronic acid to give the desired product (10.0 mg, 0.030 mmol, 52%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.24 (ddd, J = 7.8, 2.1, 1.1 Hz, 1H), 7.17 (t, J = 7.7 Hz, 1H), 7.13 (t, J = 1.9 Hz, 1H), 6.92 (dt, J = 7.4, 1.4 Hz, 1H), 5.69 (ddd, J = 5.1, 2.6, 1.0 Hz, 1H), 5.55 (dd, J = 3.7, 2.2 Hz, 1H), 3.31

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

(ddd, J = 14.3, 11.9, 6.0 Hz, 1H), 3.13 (ddd, J = 10.4, 9.1, 6.5 Hz, 1H), 3.11−3.02 (m, 5H), 2.78 (tdd, J = 12.5, 6.3, 3.9 Hz, 1H), 2.72−2.64 (m, 1H), 2.57 (dddd, J = 18.9, 11.7, 7.1, 2.6 Hz, 2H), 2.44 (dd, J = 11.4, 3.9 Hz, 1H), 2.06−2.00 (m, 1H), 1.97 (dt, J = 18.9, 5.4 Hz, 1H), 1.79 (ddtd, J = 17.4, 8.7, 3.6, 1.7 Hz, 1H), 1.45 (t, J = 11.8 Hz, 1H), 1.29 (s, 9H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 150.5, 143.1, 143.0, 139.5, 127.6, 126.3, 126.2, 125.3, 123.6, 121.0, 73.8, 65.0, 56.0, 46.2, 42.7, 40.3, 34.7, 31.9, 31.4, 28.2, 19.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{23}H_{32}NO$, 338.2478; Found, 338.2477. **Rf** = 0.34 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]D^{25}$ = +105.0° (c 1.0, $CHCl_3$). >99% *ee.*

*(10R,11aS)-1-(3-(Tert-Butyl)phenyl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-34**).* The title compound was derived from **epi-30** and (3-(*tert*-butyl)phenyl)boronic acid to give the desired product (8.80 mg, 0.026 mmol, 42% crude yield) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.24 (d, J = 0.9 Hz, 1H), 7.21−7.14 (m, 2H), 6.98 (dt, J = 7.6, 1.4 Hz, 1H), 5.74 (dd, J = 5.5, 2.3 Hz, 1H), 5.64 (d, J = 8.0 Hz, 1H), 3.37−3.30 (m, 2H), 3.28 (s, 3H), 3.22−3.08 (m, 3H), 2.71 (tdd, J = 13.4, 6.4, 3.3 Hz, 1H), 2.59 (dddd, J = 18.7, 11.8, 6.8, 2.3 Hz, 1H), 2.48 (ddd, J = 14.6, 8.0, 4.9 Hz, 1H), 2.34 (dd, J = 14.2, 3.6 Hz, 1H), 2.17 (dddd, J = 11.9, 7.7, 4.0, 2.3 Hz, 1H), 2.00 (dt, J = 18.7, 5.5 Hz, 1H), 1.61 (dd, J = 14.3, 11.0 Hz, 1H), 1.28 (s, 9H), 1.15−1.07 (m, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 150.3, 142.8, 140.3, 139.7, 127.6, 126.8, 126.0, 124.8, 124.1, 121.5, 74.9, 66.1, 56.3, 46.2, 40.0, 37.1, 34.7, 31.4, 29.3, 27.9, 18.9. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{23}H_{32}NO$, 338.2478; Found, 338.2482. **Rf** = 0.29 (silica gel, 7:1 EtOAc/MeOH). $[\alpha]D^{25}$ = +60.9° (c 0.09, $CHCl_3$). >99% *ee. Note: NMR contains 10% of an unknown impurity.*

*(10S,11aS)-1-(Benzo[d][1,3]dioxol-5-yl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**35**).* The title compound was derived from **30** and benzo[d][1,3]dioxol-5-ylboronic acid to give the desired product (11.0 mg, 0.034 mmol, 60%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 6.70 (d, J = 7.8 Hz, 1H), 6.62−6.57 (m, 2H), 5.93 (dd, J = 8.2, 1.5 Hz, 2H), 5.64 (dd, J = 5.2, 2.6 Hz, 1H), 5.59−5.54 (m, 1H), 3.26 (ddd, J = 14.3, 11.9, 6.0 Hz, 1H), 3.13 (s, 3H), 3.10−3.00 (m, 3H), 2.98 (dddd, J = 15.6, 8.8, 6.6, 3.5 Hz, 1H), 2.65−2.50 (m, 3H), 2.41 (dd, J = 11.6, 3.8 Hz, 1H), 2.18−2.12 (m, 1H), 1.94 (dt, J = 18.9, 5.2 Hz, 1H), 1.83 (ddtd, J = 17.4, 8.8, 3.5, 1.8 Hz, 1H), 1.48 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 147.0, 146.5, 142.1, 139.0, 137.7, 125.7, 122.5, 121.3, 109.6, 107.8, 101.0, 73.9, 64.9, 56.0, 46.0, 42.6, 40.1, 31.9, 28.2, 19.8. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{24}NO_3$, 326.1751; Found, 326.1754. **Rf** = 0.21 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +104.1° (c 1.0, $CHCl_3$). >99% *ee.*

*(10R,11aS)-1-(Benzo[d][1,3]dioxol-5-yl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-35**).* The title compound was derived from **epi-30** and benzo[d][1,3]dioxol-5-ylboronic acid to give the desired product (11.0 mg, 0.034 mmol, 60%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 6.68−6.66 (m, 2H), 6.64 (dd, J = 7.8, 1.7 Hz, 1H), 5.91 (dd, 2H), 5.67−5.65 (m, 1H), 5.59−5.56 (m, 1H), 3.29 (s, 3H), 3.29−3.23 (m, 2H), 3.08−2.98 (m, 3H), 2.65−2.58 (m, 1H), 2.54 (dddd, J = 18.6, 11.7, 6.8, 2.5 Hz, 1H), 2.42−2.36 (m, 1H), 2.28 (ddd, J = 14.1, 5.6, 2.3 Hz, 1H), 2.23−2.17 (m, 1H), 1.87 (dt, J = 18.7, 5.4 Hz, 1H), 1.57 (dd, J = 14.0, 11.0 Hz, 1H), 1.27−1.23 (m, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 146.8, 146.5, 142.8, 141.2, 135.7, 125.5, 122.8, 120.1, 109.9, 107.6, 100.9, 75.5, 65.3, 56.2, 46.1, 39.9, 37.7, 29.7, 28.1, 18.9. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{24}NO_3$, 326.1751; Found, 326.1754. **Rf** = 0.20 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +43.3° (c 0.9, $CHCl_3$). >99% *ee.*

*Methyl 5-((10S,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indol-1-yl)picolinate (**36**).* The title compound was derived from **30** and (6-(methoxycarbonyl)pyridin-3-yl)boronic acid to give the desired product (5.00 mg, 0.015 mmol, 29%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.51 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.58 (dd, J = 8.1, 2.2 Hz, 1H), 5.81−5.77 (m, 1H), 5.61−5.59 (m, 1H), 4.01 (s, 3H), 3.38−3.29 (m, 1H), 3.12−3.03 (m, 6H), 2.79 (tdd, J = 12.4, 6.1, 3.9 Hz, 1H), 2.64−2.53 (m, 3H), 2.45 (dd, J = 11.6, 4.0 Hz, 1H), 2.13−2.07 (m, 1H), 2.03−2.00 (m, 1H), 1.83 (ddt, J = 17.4, 8.6, 3.0 Hz, 1H), 1.53 (t, J = 12.0 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 165.8, 149.7, 146.1, 141.0, 139.0, 138.7, 136.9, 129.5, 124.3, 121.9, 73.7, 64.6, 56.2, 53.1, 46.2, 42.7, 39.9, 31.7, 28.1, 19.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{25}N_2O_3$, 341.1860; Found, 341.1864. **Rf** = 0.11 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +80.3° (c 0.5, $CHCl_3$). >99% *ee.*

*Methyl 5-((10R,11aS)-10-Methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indol-1-yl)picolinate (**epi-36**).* The title compound was derived from **epi-30** and (6-(methoxycarbonyl)pyridin-3-yl)boronic acid to give the desired product (2.70 mg, 0.008 mmol, 13%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.51 (d, J = 2.3 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.66 (dd, J = 8.1, 2.2 Hz, 1H), 5.81−5.79 (m, 1H), 5.61−5.58 (m, 1H), 4.00 (s, 3H), 3.34−3.24 (m, 1H), 3.23 (s, 3H), 3.07−3.03 (m, 3H), 2.65−2.54 (m, 2H), 2.48−2.42 (m, 1H), 2.29 (dd, J = 14.3, 5.4 Hz, 1H), 2.23−2.18 (m, 1H), 1.94 (dt, J = 19.1, 5.4 Hz, 1H), 1.50 (dd, J = 14.2, 10.4 Hz, 1H), 1.13−1.08 (m, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 165.9, 150.1, 146.2, 141.0, 140.8, 139.8, 137.1, 128.4, 124.2, 120.6, 74.9, 64.6, 56.2, 53.0, 46.1, 39.8, 38.3, 29.7, 28.1, 18.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{20}H_{25}N_2O_3$, 341.1860; Found, 341.1867. **Rf** = 0.10 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = not enough material for a reliable measurement.

*(10S,11aS)-10-Methoxy-1-(1-methyl-1H-pyrazol-4-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**37**).* The title compound was derived from **30** and (1-methyl-1*H*-pyrazol-4-yl)boronic acid to give the desired product (12.0 mg, 0.042 mmol, 74%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.35 (s, 1H), 7.21 (s, 1H), 5.72 (t, J = 3.8 Hz, 1H), 5.61 (dt, J = 4.0, 1.9 Hz, 1H), 3.84 (s, 3H), 3.31−3.21 (m, 2H), 3.16 (s, 3H), 3.06−2.96 (m, 3H), 2.56−2.46 (m, 3H), 2.39 (dd, J = 11.3, 4.2 Hz, 1H), 2.34−2.29 (m, 1H), 1.96−1.90 (m, 2H), 1.49 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 140.2, 138.3, 132.5, 127.9, 124.3, 124.2, 120.4, 74.1, 64.2, 56.0, 46.1, 42.6, 39.9, 39.0, 31.9, 27.9, 19.7. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{17}H_{24}N_3O$, 286.1914; Found, 286.1917. **Rf** = 0.09 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +44.3° (c 1.0, $CHCl_3$). >99% *ee.*

*(10R,11aS)-10-Methoxy-1-(1-methyl-1H-pyrazol-4-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-37**).* The title compound was derived from **epi-30** and (1-methyl-1*H*-pyrazol-4-yl)boronic acid to give the desired product (6.30 mg, 0.022 mmol, 33%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.42 (s, 1H), 7.31 (s, 1H), 5.85−5.83 (m, 1H), 5.63−5.61 (m, 1H), 3.82 (s, 3H), 3.37−3.33 (m, 1H), 3.32 (s, 3H), 3.24 (ddd, J = 14.4, 11.5, 5.7 Hz, 1H), 3.00−2.95 (m, 3H), 2.58−2.46 (m, 2H), 2.38 (dddd, J = 14.8, 8.0, 4.3, 2.3 Hz, 1H), 2.32−2.26 (m, 2H), 1.84 (dt, J = 18.9, 5.4 Hz, 1H), 1.72−1.66 (m, 1H), 1.51 (dd, J = 14.0, 11.4 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 142.7, 138.4, 133.8, 127.9, 123.7, 121.8, 119.0, 75.3, 64.5, 56.2, 45.9, 39.9, 39.0, 37.2, 30.1, 28.1, 18.8. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{17}H_{24}N_3O$, 286.1914; Found, 286.1921. **Rf** = 0.10 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +77.1° (c 0.55, $CHCl_3$). >99% *ee.*

*(10S,11aS)-10-Methoxy-1-(1-methyl-1H-imidazole-5-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**38**).* The title compound was derived from **30** and 1-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-imidazole to give the desired product (6.00 mg, 0.021 mmol, 37%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.26 (s, 1H), 6.53 (s, 1H), 5.59−5.55 (m, 1H), 5.43−5.39 (m, 1H), 3.39 (s, 3H), 3.14 (ddd, J = 14.4, 11.7, 5.9 Hz, 1H), 2.98 (s, 3H), 2.93 (dd, J = 14.5, 7.1 Hz, 1H), 2.89−2.85 (m, 2H), 2.49−2.32 (m, 4H), 2.16 (dd, J = 11.7, 4.0 Hz, 1H), 2.07 (dd, J = 17.7, 4.6 Hz, 1H), 1.87 (dt, J = 19.4, 5.4 Hz, 1H), 1.69−1.61 (m, 1H), 1.39 (t, J = 12.0 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 138.3, 137.6, 131.8, 130.4, 130.1, 128.7, 121.3, 73.9, 64.8, 56.1, 46.2, 42.6, 40.0, 31.9, 31.7, 27.8, 19.5. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{17}H_{24}N_3O$, 286.1914; Found, 286.1914. **Rf** = 0.1 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +31.9° (c 0.6, $CHCl_3$). >99% *ee.*

*(10S,11aS)-10-Methoxy-1-((E)-4-(trifluoromethyl)styryl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**39**).* The title compound was derived from **30** and (*E*)-(3-(trifluoromethyl)styryl)-boronic acid to give the desired product (11.0 mg, 0.029 mmol, 52%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.55 (d, J = 8.1 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 6.68 (d, J = 16.0 Hz, 1H), 6.60 (d, J = 16.0 Hz, 1H),

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

6.11 (t, J = 4.0 Hz, 1H), 5.69−5.64 (m, 1H), 3.80 (tdd, J = 12.5, 6.3, 4.0 Hz, 1H), 3.33−3.24 (m, 4H), 3.05 (dd, J = 14.5, 7.4 Hz, 1H), 2.98 (m, 1H), 2.89 (m, 1H), 2.63−2.53 (m, 2H), 2.51−2.36 (m, 3H), 2.09−2.02 (m, 1H), 1.99 (dt, J = 19.9, 5.5 Hz, 1H), 1.52 (t, J = 11.8 Hz, 1H). **^{13}C NMR{1H}** (151 MHz, $CDCl_3$): δ 141.3, 139.9, 138.0, 132.1, 129.1 (q, J = 32.4 Hz), 126.5, 125.9, 125.7 (q, J = 3.8 Hz), 124.5 (d, J = 271.8 Hz), 124.0, 119.6, 73.8, 64.1, 56.2, 46.1, 42.2, 39.9, 32.1, 27.5, 20.0. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{22}H_{25}F_3NO$, 376.1883; Found, 376.1891. **Rf** = 0.33 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +85.7° (c 1.0, $CHCl_3$). >99% *ee*.

*(10R,11aS)-10-Methoxy-1-((E)-4-(trifluoromethyl)styryl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-39**)*. The title compound was derived from **epi-30** and (*E*)-(3-(trifluoromethyl)-styryl)boronic acid to give the desired product (12.0 mg, 0.032 mmol, 54%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.52 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 6.73 (d, J = 16.1 Hz, 1H), 6.58 (d, J = 16.1 Hz, 1H), 6.14−6.12 (m, 1H), 5.68−5.65 (m, 1H), 3.43 (tt, J = 9.8, 4.9 Hz, 1H), 3.34 (s, 3H), 3.25 (ddd, J = 14.1, 11.2, 5.6 Hz, 1H), 3.03−2.97 (m, 2H), 2.90 (ddd, J = 10.9, 9.4, 4.5 Hz, 1H), 2.60−2.51 (m, 2H), 2.44−2.38 (m, 1H), 2.32−2.26 (m, 2H), 2.04−1.97 (m, 1H), 1.93 (dt, J = 19.5, 5.5 Hz, 1H), 1.66 (dd, J = 14.2, 10.5 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 141.9, 141.2, 139.0, 130.4, 128.8 (q, J = 32.4 Hz), 127.0, 126.7, 126.5, 126.3, 125.5 (q, J = 3.8 Hz), 124.3 (q, J = 271.7 Hz), 124.1, 118.4, 75.1, 63.6, 56.1, 45.8, 39.8, 37.8, 30.5, 27.6, 19.0. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{22}H_{25}F_3NO$, 376.1883; Found, 376.1886. **Rf** = 0.23 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +96.5° (c 0.4, $CHCl_3$). >99% *ee*.

*(10S,11aS)-10-Methoxy-1-(1-methyl-1H-indol-3-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**40**)*. The title compound was derived from **30** and 1-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole to give the desired product (17.0 mg, 0.051 mmol, 90%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.67 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 6.80 (s, 1H), 5.88−5.84 (m, 1H), 5.63−5.58 (m, 1H), 3.74 (s, 3H), 3.43 (ddd, J = 14.3, 11.8, 6.1 Hz, 1H), 3.18−3.07 (m, 3H), 2.90−2.83 (m, 4H), 2.71−2.63 (m, 1H), 2.63−2.54 (m, 2H), 2.51 (dd, J = 11.3, 3.9 Hz, 1H), 2.13 (dt, J = 18.9, 5.4 Hz, 1H), 2.09−2.06 (m, 1H), 1.88−1.80 (m, 1H), 1.48 (t, J = 11.7 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 139.7, 136.5, 133.3, 128.1, 126.7, 125.4, 121.8, 120.9, 119.9, 119.4, 116.4, 109.1, 74.1, 65.3, 55.8, 46.2, 42.3, 40.2, 32.9, 32.1, 28.0, 19.7. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{22}H_{27}N_2O$, 335.2118; Found, 335.2123. **Rf** = 0.24 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +172.4° (c 1.0, $CHCl_3$). >99% *ee*.

*(10R,11aS)-10-Methoxy-1-(1-methyl-1H-indol-3-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-40**)*. The title compound was derived from **epi-30** and 1-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indole to give the desired product (11.6 mg, 0.035 mmol, 63%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.62 (d, J = 7.9 Hz, 1H), 7.25 (d, J = 3.7 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 7.10−7.07 (m, 2H), 5.99−5.97 (m, 1H), 5.64 (d, J = 7.8 Hz, 1H), 3.72 (s, 3H), 3.42−3.35 (m, 2H), 3.27 (s, 3H), 3.15−3.06 (m, 3H), 2.71−2.60 (m, 2H), 2.40−2.35 (m, 2H), 2.31−2.26 (m, 1H), 2.08−2.02 (m, 1H), 1.57−1.52 (m, 2H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 142.0, 136.7, 134.6, 128.7, 126.2, 125.4, 121.7, 120.0, 119.8, 119.3, 114.3, 109.1, 75.2, 65.7, 56.2, 46.1, 40.1, 37.2, 32.9, 30.0, 28.2, 18.9. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{22}H_{27}N_2O$, 335.2118; Found, 335.2118. **Rf** = 0.17 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +200.9° (c 0.22, $CHCl_3$). >99% *ee*.

*(10S,11aS)-1-(Benzo[b]thiophen-3-yl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**41**)*. The title compound was derived from **30** and benzo[*b*]thiophen-3-ylboronic acid to give the desired product (9.60 mg, 0.028 mmol, 50%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.83 (dddd, J = 15.2, 8.0, 1.2, 0.7 Hz, 2H), 7.41−7.31 (m, 2H), 6.99 (s, 1H), 5.84 (dd, J = 4.7, 2.8 Hz, 1H), 5.61 (d, J = 3.3 Hz, 1H), 3.46 (ddd, J = 14.3, 11.9, 6.0 Hz, 1H), 3.22−3.14 (m, 3H), 2.77−2.67 (m, 4H), 2.67−2.57 (m, 2H), 2.56−2.47 (m, 2H), 2.17 (dt, J = 19.2, 5.5 Hz, 1H), 2.08−2.00 (m, 1H), 1.84−1.76 (m, 1H), 1.54 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 139.7, 139.7, 138.3, 136.6, 134.5, 127.5, 124.4, 124.4, 123.5, 122.9, 122.8, 122.2, 73.6, 65.7, 55.9, 46.3, 41.6, 40.2, 32.0, 27.9, 19.4. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{21}H_{24}NOS$, 338.1573; Found, 338.1584. **Rf** = 0.23 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +68.7° (c 0.96, $CHCl_3$). >99% *ee*.

*(10R,11aS)-1-(Benzo[b]thiophen-3-yl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-41**)*. The title compound was derived from **epi-30** and benzo[*b*]thiophen-3-ylboronic acid to give the desired product (4.50 mg, 0.013 mmol, 24%) **^{1}H NMR** (600 MHz, C_6D_6): δ 7.79 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.21 (t, J = 7.6 Hz, 1H), 7.13 (s, 1H), 7.08 (t, J = 7.5 Hz, 1H), 5.68−5.65 (m, 1H), 5.31−5.28 (m, 1H), 3.39−3.33 (m, 1H), 3.23 (ddd, J = 14.3, 11.6, 5.5 Hz, 1H), 3.02 (s, 3H), 3.00−2.95 (m, 1H), 2.87 (td, J = 10.1, 9.5, 4.5 Hz, 1H), 2.79 (dd, J = 14.5, 6.7 Hz, 1H), 2.54−2.47 (m, 2H), 2.26−2.19 (m, 1H), 2.18−2.13 (m, 1H), 2.10−2.04 (m, 1H), 1.74 (dd, J = 13.9, 10.7 Hz, 1H), 1.51 (dt, J = 18.7, 5.4 Hz, 1H), 1.39−1.34 (m, 1H). **^{13}C{1H} NMR** (151 MHz, C_6D_6): δ 142.0, 140.9, 140.1, 137.0, 136.3, 128.4, 124.5, 124.3, 123.2, 123.0, 122.9, 120.2, 75.7, 65.7, 55.7, 46.5, 40.3, 38.8, 30.0, 28.7, 18.7. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{21}H_{24}NOS$, 338.1573; Found, 338.1572. **Rf** = 0.17 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +91.6° (c 0.24, $CHCl_3$). >99% *ee*.

*(10S,11aS)-10-Methoxy-1-(thiophen-3-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**42**)*. The title compound was derived from **30** and thiophen-3-ylboronic acid to give the desired product (12.5 mg, 0.044 mmol, 77%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.17 (dd, J = 5.0, 3.0 Hz, 1H), 7.04 (dd, J = 3.0, 1.2 Hz, 1H), 6.94 (dd, J = 5.0, 1.3 Hz, 1H), 5.81−5.79 (m, 1H), 5.60−5.57 (m, 1H), 3.28 (ddd, J = 14.3, 11.8, 6.1 Hz, 1H), 3.11 (s, 3H), 3.08−2.98 (m, 3H), 2.92 (dddd, J = 12.5, 8.8, 6.2, 3.9 Hz, 1H), 2.64−2.50 (m, 3H), 2.40 (dd, J = 11.4, 3.9 Hz, 1H), 2.26−2.18 (m, 1H), 1.94 (dt, J = 19.1, 5.4 Hz, 1H), 1.86 (dddd, J = 17.4, 8.7, 3.5, 1.9 Hz, 1H), 1.46 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 144.1, 139.9, 137.2, 128.7, 125.6, 124.6, 121.6, 120.8, 74.1, 64.5, 56.1, 46.1, 43.0, 40.1, 32.0, 28.0, 19.7. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{17}H_{22}NOS$, 288.1417; Found, 288.1420. **Rf** = 0.18 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +50.2° (c 0.5, $CHCl_3$). >99% *ee*.

*(10R,11aS)-10-Methoxy-1-(thiophen-3-yl)-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-42**)*. The title compound was derived from **epi-30** and thiophen-3-ylboronic acid to give the desired product (11.0 mg, 0.038 mmol, 45%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.18 (s, 1H), 7.16−7.14 (m, 1H), 7.02 (d, J = 5.0 Hz, 1H), 5.90−5.88 (m, 1H), 5.63−5.60 (m, 1H), 3.36−3.24 (m, 5H), 3.04−2.98 (m, 3H), 2.60−2.53 (m, 2H), 2.37−2.28 (m, 3H), 1.89 (dt, J = 18.9, 5.5 Hz, 1H), 1.56 (dd, J = 14.3, 11.5 Hz, 1H), 1.53−1.48 (m, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 142.0, 141.6, 137.9, 128.5, 125.4, 124.3, 121.8, 119.6, 75.3, 64.8, 56.2, 46.0, 39.9, 37.5, 29.8, 28.1, 18.91. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{17}H_{22}NOS$, 288.1417; Found, 288.1426. **Rf** = 0.21 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +83.9° (c 0.03, $CHCl_3$). >99% *ee*.

*(10S,11aS)-1-((E)-3-Fluorostyryl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**43**)*. The title compound was derived from **30** and (*E*)-(3-fluorostyryl)boronic acid to give the desired product (17.0 mg, 0.052 mmol, 92%) **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.26 (td, J = 8.0, 6.0 Hz, 1H), 7.09 (d, J = 7.7 Hz, 1H), 7.03 (dt, J = 10.3, 2.1 Hz, 1H), 6.94−6.87 (m, 1H), 6.62 (d, J = 15.9 Hz, 1H), 6.51 (d, J = 15.9 Hz, 1H), 6.07 (t, J = 4.0 Hz, 1H), 5.67−5.64 (m, 1H), 3.81 (dddd, J = 12.6, 8.8, 6.4, 4.0 Hz, 1H), 3.26 (s, 4H), 3.05 (dd, J = 14.4, 7.3 Hz, 1H), 2.98 (td, J = 9.0, 3.8 Hz, 1H), 2.89 (td, J = 9.5, 9.1, 6.6 Hz, 1H), 2.62−2.52 (m, 2H), 2.50−2.44 (m, 1H), 2.43−2.36 (m, 2H), 2.08−2.01 (m, 1H), 2.01−1.94 (m, 1H), 1.52 (t, J = 11.8 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 163.3 (d, J = 245.0 Hz), 140.2 (d, J = 7.7 Hz), 137.9, 130.8, 130.2 (d, J = 8.3 Hz), 126.3 (d, J = 2.7 Hz), 123.3, 122.4 (d, J = 2.8 Hz), 119.6, 114.2 (d, J = 21.5 Hz), 112.7 (d, J = 21.9 Hz), 73.8, 64.1, 56.2, 46.2, 42.2, 39.9, 32.0, 27.4, 20.0. **HRMS (ESI)** m/z: [M + H]$^+$ Calcd for $C_{21}H_{25}NOF$, 326.1915; Found, 326.1923. **Rf** = 0.35 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]_D^{25}$ = +41.3° (c 1.0, $CHCl_3$). >99% *ee*.

*(10R,11aS)-1-((E)-3-Fluorostyryl)-10-methoxy-3,4,7,9,10,11-hexahydro-6H-pyrido[2,1-i]indole (**epi-43**)*. The title compound was derived from **epi-30** and (*E*)-(3-fluorostyryl)boronic acid to give the desired product (6.50 mg, 0.020 mmol, 35%). **^{1}H NMR** (600 MHz,

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

$CDCl_3$): δ 7.23 (td, J = 8.0, 6.0 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H), 7.03 (dt, J = 10.4, 2.1 Hz, 1H), 6.88 (td, J = 8.5, 2.6 Hz, 1H), 6.67 (d, J = 16.1 Hz, 1H), 6.49 (d, J = 16.1 Hz, 1H), 6.10−6.06 (m, 1H), 5.68−5.63 (m, 1H), 3.41 (tt, J = 9.8, 4.7 Hz, 1H), 3.35 (s, 3H), 3.24 (ddd, J = 14.3, 11.4, 5.7 Hz, 1H), 3.02−2.93 (m, 2H), 2.90 (ddd, J = 11.0, 9.4, 4.5 Hz, 1H), 2.61−2.48 (m, 2H), 2.45−2.36 (m, 1H), 2.32−2.24 (m, 2H), 2.08−1.95 (m, 1H), 1.90 (dt, J = 19.4, 5.4 Hz, 1H), 1.64 (dd, J = 14.2, 10.7 Hz, 1H). **^{13}C{1H} NMR** (151 MHz, $CDCl_3$): δ 163.3 (d, J = 245.0 Hz), 142.3, 140.3 (d, J = 7.7 Hz), 139.3, 130.1 (d, J = 8.7 Hz), 129.4, 126.9 (d, J = 2.7 Hz), 123.7, 122.4 (d, J = 2.6 Hz), 118.2, 114.0 (d, J = 21.5 Hz), 112.6 (d, J = 21.6 Hz), 75.4, 63.7, 56.2, 45.9, 39.9, 38.0, 30.6, 27.8, 19.1. **HRMS (ESI)** m/z: $[M + H]^+$ Calcd for $C_{21}H_{25}NOF$, 326.1915; Found, 326.1917. **Rf** = 0.33 (silica gel, 6:2 EtOAc/MeOH). $[\alpha]D^{25}$ = +74.4° (c 0.54, $CHCl_3$). >99% *ee*.

Pharmacology. *Materials.* (*S*)-Nicotine and all chemicals for the buffers were purchased from Sigma-Aldrich (St. Louis, MO), and Dulbecco's Modified Eagle Medium Glutamax-I (DMEM) medium, serum, Hanks Buffered Salt Solution (HBSS), and antibiotics were obtained from Invitrogen (Paisley, UK). [^{3}H]epibatidine and the Fluo-4/AM dye were purchased from PerkinElmer (Waltham, MA) and Molecular Probes (Eugene, OR), respectively. The stable hα4β2- and hα6/α3β2β3$^{V9'S}$-HEK293 cell lines were obtained from Dr. Tino Dyhring (Saniona A/S, Ballerup, Denmark), and the stable rα3β4- and rα4β4-HEK293 cell lines were obtained from Dr. Kenneth J. Kellar (Georgetown University Medical Center, Washington, D.C.).[2]

Cell Culture. The four nAChR-expressing HEK293 cell lines were cultured in a humidified atmosphere at 37 °C and 5% CO_2 in DMEM supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), 5% dialyzed fetal bovine serum, and, for the rα3β4- and rα4β4-HEK293 cell lines, 1 mg/mL G-418.

[^{3}H]epibatidine-Binding Assay. The binding affinities of the test compounds were determined at membranes from the hα4β2-, hα6/α3β2β3$^{V9'S}$-, rα3β4-, and rα4β4-HEK293 cell lines in a [^{3}H]-epibatidine competition binding assay, as previously described.[25a,b] Cells were collected at ∼90% confluency by scraping into ice-cold assay buffer (140 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM HEPES, pH 7.4), homogenized for 10 s, and centrifuged for 30 min at 47,810 ×g at 4 °C. The pellet was resuspended in ice-cold fresh assay buffer, homogenized, and centrifuged for another 30 min under the same conditions. The supernatant was decanted, and the cell membranes were stored at −80 °C until use. On the day of the experiment, cell membranes were resuspended in assay buffer and incubated with [^{3}H]epibatidine and various concentrations of test compounds at room temperature for 3−4 h while shaking. In the competition binding experiments, membranes were incubated with a fixed concentration of [^{3}H]-epibatidine hα4β2 and hα6/α3β2β3$^{V9'S}$: ∼30 pM (range: 20−40 pM) and rα3β4 and rα4β4: ∼150 pM (range: 100−200 pM) in the presence of various concentrations of the test compounds or (*S*)-nicotine in a total reaction volume of 3 mL. In saturation binding experiments performed in parallel, the membranes were incubated with 12 different concentrations of [^{3}H]epibatidine (spanning from 1 pM to 30 nM) in the absence (total binding) or presence of 100 μM (*S*)-nicotine (non-specific binding). In both saturation and competition binding experiments, the amount of cell membranes used and total reaction volumes were adjusted so that the bound/free ratios of the radioligand always were <10%. Prior to harvesting, Whatman GF/C filters (PerkinElmer) were treated with 0.2% polyethyleneimine solution for 30 min. The reaction was terminated by filtration through the presoaked filters using a Brandell M-48T cell harvester (Alpha Biotech, London, UK) and washed three times with 5 mL ice-cold harvesting buffer (0.9% NaCl, 10 mM Tris−HCl, pH 7.4). Filters were subsequently allowed to dry at room temperature and then transferred to scintillation vials, and 3 mL OptiFluor (PerkinElmer) was added to each sample. The filter-retained radioactivity and the exact [^{3}H]epibatidine concentrations used in the experiments were determined by liquid scintillation counting using a Tri-Carb 4910 TR (PerkinElmer). The test compounds were tested at least three times at each receptor.

Ca^{2+}/Fluo-4 Assay. The functional properties of the test compounds were characterized at the hα4β2-HEK293 and rα3β4-HEK293 cell lines in the fluorescence-based Ca^{2+}/Fluo-4 assay performed essentially, as previously described.[25b] Briefly, the cells were split into poly-D-lysine-coated black 96-well plates with clear bottom (6×10^4 cells/well). The following day the culture medium was aspirated, and the cells were incubated in 50 μL of incubation buffer (HBSS containing 20 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 2.5 mM probenecid, pH 7.4) supplemented with 6 mM Fluo−4/AM at 37 °C for 1 h. Then, the buffer was aspirated, the cells were washed once with 100 μL of incubation buffer, once with 100 μL of assay buffer (140 mM *N*-methyl-D-glucamine, 5 mM KCl, 1 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM HEPES, and 2.5 mM probenecid, pH 7.4), and then, 100 μL of assay buffer was added to the cells (in the antagonist experiments, the test compound was added to the buffer at this point). The 96-well plate was assayed in a FLEXStation^3 (Molecular Devices, Crawley, UK) measuring emission [in fluorescence units (FU)] at 525 nm caused by excitation at 485 nm before and up to 90 s after addition of 33.3 μL agonist solution in assay buffer. The compounds were initially tested as agonists at the two receptors, and none of them displayed significant agonist activity at concentrations up to 100 μM. The compounds were characterized as antagonists in duplicate at least three times at the two receptors using (*S*)-nicotine ∼ EC_{80} (range EC_{70}−EC_{90}) as an agonist.

Data Analysis. Data analysis was performed using GraphPad Prism 7.0c (GraphPad Software, Inc., La Jolla, CA). For the saturation [^{3}H]epibatidine-binding data, specific binding was analyzed with a nonlinear regression one site-specific binding model given by the equation: $Y = B_{max} \times X/(K_d + X)$, where Y is the specific [^{3}H]epibatidine binding, B_{max} is the total amount of binding, X is the [^{3}H]epibatidine concentration, and K_d is the dissociation constant. The competition binding data were analyzed with a nonlinear regression one site-fit log IC_{50} model given by the following equation: Y = bottom + (top − bottom)/(1 + 10((logIC_{50} − X)*n_H)), where Y is the specific [^{3}H]epibatidine binding, top and bottom are the plateau values of the curves, X is the test compound concentration, IC_{50} is the equilibrium affinity of the test compound, and n_H is the Hill slope. K_i values were calculated from the determined IC_{50} values using the Cheng−Prusoff equation [32]: K_i = IC_{50}/(1 − ([RL]/K_d)), where [RL] is the [^{3}H]epibatidine concentration used for the specific experiment and K_D is the dissociation constant determined in the saturation binding experiments.

The data from the Ca^{2+}/Fluo-4 assay were extracted as the difference in relative fluorescence units (ΔRFU) between the maximum fluorescence level measured after the agonist application and the basal level measured before the agonist application. The concentration−inhibition relationships of the test compounds as antagonists were fitted to a nonlinear regression curve fit with variable slope Y = bottom + (top − bottom)/(1 + 10^((logIC_{50} − X)*n_H)), where top and bottom values are plateaus in the units of the response axis, X is the concentration of the ligand, IC_{50} is the concentration of the ligand that gives a response half way between bottom and top, and n_H is the Hill slope.

Molecular Modeling. The modeling study was performed using the Drug Discovery Suite 2020−3 from Schrodinger Inc.

The structure from a study by Yu *et al.*[6b] was processed using the standard protocol for the protein preparation wizard in standard settings. A model including the water molecule seen in the X-ray structure of the AChBP (PDB: 4alx) was also made for docking in the same way. All ligands were treated with ligprep before docking, however, in the protonated state of the amine and without generating other stereoisomers or tautomers. Two grids were generated in Glide in standard settings: with and without a water molecule included. All ligands were docked with Glide in both models allowing up to five poses for each ligand. The best scoring poses in the model including water are provided in the Supporting Information. Figures were generated using Pymol 1.8.0.4.

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c00707.

Experimental details and docking poses of investigated ligands (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Jesper L. Kristensen − *Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark;* orcid.org/0000-0002-5613-1267; Email: jesper.kristensen@sund.ku.dk

Authors

Sebastian Clementson − *Molecular Discovery and Innovation, H. Lundbeck A/S, Valby 2500, Denmark; Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark*

Sergio Armentia Matheu − *Molecular Discovery and Innovation, H. Lundbeck A/S, Valby 2500, Denmark; Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark*

Emil Märcher Rørsted − *Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark;* orcid.org/0000-0002-2783-6655

Henrik Pedersen − *Molecular Discovery and Innovation, H. Lundbeck A/S, Valby 2500, Denmark*

Anders A. Jensen − *Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark*

Rasmus P. Clausen − *Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen 2100, Denmark;* orcid.org/0000-0001-9466-9431

Paulo Vital − *Molecular Discovery and Innovation, H. Lundbeck A/S, Valby 2500, Denmark;* Present Address: Current address: Department of Research & Development, BIAL-Portela & Ca, S.A., S. Mamede do Coronado, Portugal.

Emil Glibstrup − *Molecular Discovery and Innovation, H. Lundbeck A/S, Valby 2500, Denmark*

Mikkel Jessing − *Molecular Discovery and Innovation, H. Lundbeck A/S, Valby 2500, Denmark*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.1c00707

Author Contributions

Conceptualization: S.C., P.V., M.J., and J.L.K.; investigation: S.C., S.A.M., E.M.R., H.P., A.A.J., and R.P.C.; supervision: S.C., P.V., E.G., M.J., and J.L.K.; and writing: S.C., A.A.J., R.P.C., M.J., and J.L.K.

Funding

This research was financially supported by the Innovation Fund Denmark (Case no. 7038-00149B) and H. Lundbeck A/S.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Medicinal Chemistry Department at H. Lundbeck A/S for valuable resources and inputs. Drs. Tino Dyhring (Saniona A/S, Ballerup, Denmark) and Ken Kellar (Georgetown University Medical Center, Washington, DC) are thanked for their generous gifts of nAChR-expressing cell lines.

■ REFERENCES

(1) Folkers, K.; Unna, K. Erythrina alkaloids, II. A review, and new data on the alkaloids of species of the genus Erythrina. *J. Am. Pharm. Assoc.* **1938**, *27*, 693.

(2) Lehman, A. Actions of Erythrina Americana, A Possible Curare Substitute. *J. Am. Pharm. Assoc.* **1937**, *60*, 69.

(3) Garín-Aguilar, M. E.; Luna, J. E.; Soto-Hernández, M.; del Toro, G. V.; Vázquez, M. M. Effect of crude extracts of Erythrina americana Mill. on aggressive behavior in rats. *J. Ethnopharmacol.* **2000**, *69*, 189.

(4) Shapiro, S.; Baker, A. B. Treatment of paralysis agitans with dihydro-beta-erythroidine. *Am. J. Med.* **1950**, *8*, 153.

(5) Andreasen, J.; Olsen, G.; Wiborg, O.; Redrobe, J. Anti-depressant-like effects of nicotinic acetylcholine receptor antagonists, but not agonists, in the mouse forced swim and mouse tail suspension tests. *J. Psychopharmacol.* **2009**, *23*, 797.

(6) (a) Burman, M. S. Therapeutic use of curare and erythroidine hydrochloride for spastic and dystonic states. *Arch. Neurol. Psychiatr.* **1938**, *41*, 307. (b) Yu, R.; Tae, H. S.; Xu, Q.; Craik, D. J.; Adams, D. J.; Jiang, T.; Kaas, Q. Molecular dynamics simulations of dihydro-β-erythroidine bound to the human α4β2 nicotinic acetylcholine receptor. *Br. J. Pharmacol.* **2019**, *176*, 2750. (c) Shahsavar, A.; Kastrup, J. S.; Nielsen, E.Ø.; Kristensen, J. L.; Gajhede, M.; Balle, T. Crystal Structure of Lymnaea stagnalis AChBP Complexed with the Potent nAChR Antagonist DHβE Suggests a Unique Mode of Antagonism. *PLoS One* **2012**, *7*, 1.

(7) Majinda, R. R.. In *Progress in the Chemistry of Organic Natural Products*; Kinghorn, D. A., Falk, H., Gibbons, S., Kobayashi, J., Asakawa, Y., Liu, J.-K., Eds.; Springer: Cham, 2018, pp 95−152.

(8) Reimann, E.. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Falk, H., Kirby, G., Eds.; Springer: Vienna, 2007, pp 2−56.

(9) Boekelheide, V.; Weinstock, J.; Grundon, M. F.; Sauvage, G. L.; Angello, E. J. The Structure of β-Erythroidine and its Derivatives. *J. Am. Chem. Soc.* **1953**, *75*, 2550.

(10) (a) Parsons, A. F.; Palframan, M. J. Erythrina and Related Alkaloids. *Alkaloids*; Cordell, G. A., Ed.; Elsevier: Heslington, York, 2010; Vol. *68*, pp 39−81. (b) Heller, S. T.; Kiho, T.; Narayan, A. R. H.; Sarpong, R. Protic-Solvent-Mediated Cycloisomerization of Quinoline and Isoquinoline Propargylic Alcohols: Syntheses of (±)-3-Demethoxyerythratidinone and (±)-Cocculidine. *Angew. Chem., Int. Ed.* **2013**, *52*, 11129. (c) Kawasaki, T.; Onoda, N.; Watanabe, H.; Kitahara, T. Total synthesis of (±)-cocculolidine. *Tetrahedron Lett.* **2001**, *42*, 8003. (d) Funk, R. L.; Belmar, J. Total synthesis of (±)-isophellibiline. *Tetrahedron Lett.* **2012**, *53*, 176. (e) Jepsen, T. H.; Glibstrup, E.; Crestey, F.; Jensen, A. A.; Kristensen, J. L. A strategic approach to [6,6]-bicyclic lactones: application towards the CD fragment of DhβE. *Beilstein J. Org. Chem.* **2017**, *13*, 988. (f) Jepsen, T. H.; Jensen, A. A.; Lund, M. H.; Glibstrup, E.; Kristensen, J. L. Synthesis and Pharmacological Evaluation of DHβE Analogues as Neuronal Nicotinic Acetylcholine Receptor Antagonists. *ACS Med. Chem. Lett.* **2014**, *5*, 766. (g) Chuang, K. V.; Navarro, R.; Reisman, S. E. Benzoquinone-derived sulfinylimines as versatile intermediates for alkaloid synthesis: Total synthesis of (−)-3-demethoxyerythratidinone. *Chem. Sci.* **2011**, *2*, 1086. (h) Blackham, E. E.; Booker-Milburn, K. I. A Short Synthesis of (±)-3-Demethoxyerythratidinone by Ligand-Controlled Selective Heck Cyclization of Equilibrating Enamines. *Angew. Chem., Int. Ed.* **2017**, *56*, 6613. (i) Crestey, F.; Jensen, A. A.; Borch, M.; Andreasen, J. T.; Andersen, J.; Balle, T.; Kristensen, J. L. Design, synthesis, and biological evaluation of Erythrina alkaloid analogues as neuronal

nicotinic acetylcholine receptor antagonists. *J. Med. Chem.* **2013**, *56*, 9673. (j) Crestey, F.; Jensen, A. A.; Soerensen, C.; Magnus, C. B.; Andreasen, J. T.; Peters, G. H. J.; Kristensen, J. L. Dual Nicotinic Acetylcholine Receptor $\alpha4\beta2$ Antagonists/$\alpha7$ Agonists: Synthesis, Docking Studies, and Pharmacological Evaluation of Tetrahydroisoquinolines and Tetrahydroisoquinolinium Salts. *J. Med. Chem.* **2018**, *61*, 1719. (k) Clementson, S.; Jessing, M.; Pedersen, H.; Vital, P.; Kristensen, J. L. Enantioselective Total Synthesis of (+)-Dihydro-β-erythroidine. *J. Am. Chem. Soc.* **2019**, *141*, 8783. (l) Clementson, S.; Jessing, M.; Vital, P. J.; Kristensen, J. L. Development of a Divergent Route to Erythrina Alkaloids. *Synlett* **2020**, *31*, 327. (m) Umihara, H.; Yoshino, T.; Shimokawa, J.; Kitamura, M.; Fukuyama, T. Development of a Divergent Synthetic Route to the Erythrina Alkaloids: Asymmetric Syntheses of 8-Oxo-erythrinine, Crystamidine, 8-Oxo-erythraline, and Erythraline. *Angew. Chem., Int. Ed.* **2016**, *55*, 6915.

(11) (a) Fukumoto, H.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. Total Synthesis of (+)-β-Erythroidine. *Angew. Chem., Int. Ed.* **2006**, *45*, 2731.

(12) (a) Trost, B. M.; Schäffner, B.; Osipov, M.; Wilton, D. A. A. Palladium-Catalyzed Decarboxylative Asymmetric Allylic Alkylation of β-ketoesters: An Unusual Counterion Effect. *Angew. Chem., Int. Ed.* **2011**, *50*, 3548. (b) Trost, B. M.; Crawley, M. L. Asymmetric Transition-Metal-Catalyzed Allylic Alkylations: Applications in Total Synthesis. *Chem. Rev.* **2003**, *103*, 2921.

(13) Vanrheenen, V.; Kelly, R. C.; Cha, D. Y.; Company, T. U. An improved catalytic OsO4 oxidation of olefins to cis-1, 2-glycols using tertiary amine oxides as the oxidant. *Tetrahedron Lett.* **1976**, *17*, 1973.

(14) (a) Pappo, R.; Allen, D., Jr.; Lemieux, R.; Johnson, W. Notes—Osmium Tetroxide-Catalyzed Periodate Oxidation of Olefinic Bonds. *J. Org. Chem.* **1956**, *21*, 478. (b) Li, W.; Chen, Z.; Yu, D.; Peng, X.; Wen, G.; Wang, S.; Xue, F.; Liu, X.-Y.; Qin, Y. Asymmetric Total Syntheses of the Akuammiline Alkaloids (−)-Strictamine and (−)-Rhazinoline. *Angew. Chem., Int. Ed.* **2019**, *58*, 6059.

(15) (a) Hartley, R. C.; Li, J.; Main, C. A.; Mckiernan, G. J. Titanium carbenoid reagents for converting carbonyl groups into alkenes. *Tetrahedron* **2007**, *63*, 4825. (b) Takai, K.; Hotta, Y.; Oshima, K.; Nozaki, H. Effective methods of carbonyl methylenation using CH2I2-Zn-Me3Al and CH2Br2-Zn-TiCl4 system. *Tetrahedron Lett.* **1978**, *19*, 2417. (c) Okazoe, T.; Hibino, J.-i.; Takai, K.; Nozaki, H. Chemoselective methylenation with a methylenedianion synthon. *Tetrahedron Lett.* **1985**, *26*, 5581. (d) Hibino, J.-i.; Okazoe, T.; Takai, K.; Nozaki, H. Carbonyl methylenation of easily enolizable ketones. *Tetrahedron Lett.* **1985**, *26*, 5579. (e) Tebbe, F. N.; Parshall, G. W.; Reddy, G. S. Olefin homologation with titanium methylene compounds. *J. Am. Chem. Soc.* **1978**, *100*, 3611. (f) Petasis, N. A.; Bzowej, E. I. Titanium-mediated carbonyl olefinations. 1. Methylenations of carbonyl compounds with dimethyltitanocene. *J. Am. Chem. Soc.* **1990**, *112*, 6392. (g) Yan, T.-H.; Tsai, C.-C.; Chien, C.-T.; Cho, C.-C.; Huang, P.-C. Dichloromethane Activation. Direct Methylenation of Ketones and Aldehydes with CH2Cl2 Promoted by Mg/TiCl4/THF. *Org. Lett.* **2004**, *6*, 4961.

(16) (a) Van Staden, L. F.; Gravestock, D.; Ager, D. J. New developments in the Peterson olefination reaction. *Chem. Soc. Rev.* **2002**, *31*, 195. (b) Chen, D.; Liu, H.-M.; Li, M.-M.; Yan, Y.-M.; Xu, W.-D.; Li, X.-N.; Cheng, Y.-X.; Qin, H.-B. Concise synthesis of (±)-Lingzhiol via epoxy-arene cyclization. *Chem. Commun.* **2015**, *51*, 14594.

(17) Kumar, T.; Jarhad, D. B.; Mobin, S. M.; Singh, V. Molecular complexity and diversity from aromatics. Intramolecular cycloaddition of cyclohexa-2,4-dienones and sigmatropic shift in excited state: a unified approach towards synthesis of polycyclic frameworks related to crotogoudin, conidiogenol, and crinipellins. *Tetrahedron* **2016**, *72*, 5377.

(18) Schuppe, A. W.; Huang, D.; Chen, Y.; Newhouse, T. R. Total synthesis of (−)-Xylogranatopyridine B via a Palladium-catalyzed oxidative stannylation of enones. *J. Am. Chem. Soc.* **2018**, *140*, 2062.

(19) Hoffmann, R. W. α-Chiral allylboronates: reagents for asymmetric synthesis. *Pure Appl. Chem.* **1988**, *60*, 123.

(20) (a) Katakam, R.; Anugula, R.; Macha, L.; Batchu, V. R. Stereoselective synthesis of N-benzyl conduramine F-1, N-benzyl ent-conduramine E-1, dihydroconduramine F-1 and ent-dihydroconduramine E-1. *Tetrahedron Lett.* **2017**, *58*, 559. (b) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. Synthesis and activity of a new generation of ruthenium-based olefin metathesis catalysts coordinated with 1, 3-dimesityl-4, 5-dihydroimidazol-2-ylidene ligands. *Org. Lett.* **1999**, *1*, 953.

(21) (a) Kuwajiama, I.; Hirokazu, U. Regioselective Arylation of Silyl Enol Ethers of Methyl Ketones with Aryl Bromides. *J. Am. Chem. Soc.* **1982**, *104*, 6831. (b) Moradi, W. A.; Buchwald, S. L. Palladium-Catalyzed α-Arylation of Esters. *J. Am. Chem. Soc.* **2001**, *123*, 7996. (c) Carfagna, C.; Musco, A.; Sallese, G.; Santi, R.; Fiorani, T. Palladium-catalyzed coupling reactions of aryl triflates or halides with ketene trimethylsilyl acetals. A new route to alkyl 2-arylalkanoates. *J. Org. Chem.* **1991**, *56*, 261. (d) Jørgensen, M.; Lee, S.; Liu, X.; Wolkowski, J. P.; Hartwig, J. F. Efficient synthesis of alpha-aryl esters by room-temperature palladium-catalyzed coupling of aryl halides with ester enolates. *J. Am. Chem. Soc.* **2002**, *124*, 12557.

(22) Shang, R.; Ji, D.-S.; Chu, L.; Fu, Y.; Liu, L. Synthesis of α-Aryl Nitriles through Palladium-Catalyzed Decarboxylative Coupling of Cyanoacetate Salts with Aryl Halides and Triflates. *Angew. Chem., Int. Ed.* **2011**, *50*, 4470.

(23) Caprioglio, D.; Fletcher, S. P. An alternative synthesis of the breast cancer drug fulvestrant (Faslodex): Catalyst control over C−C bond formation. *Chem. Commun.* **2015**, *51*, 14866.

(24) Shi, J.; Shigehisa, H.; Guerrero, C. A.; Shenvi, R. A.; Li, C.-C.; Baran, P. S. Stereodivergent Synthesis of 17-α and 17-β-Aryl Steroids: Application and Biological Evaluation of D-Ring Cortistatin Analogues. *Angew. Chem., Int. Ed.* **2009**, *48*, 4328.

(25) (a) Jensen, A. A.; Mikkelsen, I.; Frølund, B.; Bräuner-Osborne, H.; Falch, E.; Krogsgaard-Larsen, P. Carbamoylcholine homologs: novel and potent agonists at neuronal nicotinic acetylcholine receptors. *Mol. Pharmacol.* **2003**, *64*, 865. (b) Sandager-Nielsen, K.; Ahring, P. K.; Klein, J.; van Hout, M.; Thaneshwaran, S.; Dos Santos, A. B.; Jacobsen, T. A.; Amrutkar, D. V.; Peters, D.; Jensen, A. A.; Kohlmeier, K. A.; Christophersen, P.; Dyhring, T. Characterization of AN317, a novel selective agonist of $\alpha6\beta2$-containing nicotinic acetylcholine receptors. *Biochem. Pharmacol.* **2020**, *174*, 113786.

(26) Inubushi, Y.; Nomura, K.-i.; Miyawaki, M. Studies on the Alkaloids of Menispermaceous Plants. CXCVII. *J. Pharm. Soc. Jpn.* **1963**, *83*, 282.

(27) Yunusov, S. Ob Alkaloidakh Cocculus Laurifolius DC. 1. *Zh. Obshch. Khim.* **1950**, *20*, 368.

(28) Timmermann, D.; Sandager-Nielsen, K.; Dyhring, T.; Smith, M.; Jacobsen, A.-M.; Nielsen, E.; Grunnet, M.; Christensen, J.; Peters, D.; Kohlhaas, K.; Olsen, G.; Ahring, P. Augmentation of cognitive function by NS9283, a stoichiometry-dependent positive allosteric modulator of $\alpha2$-and $\alpha4$-containing nicotinic acetylcholine receptors. *Br. J. Pharmacol.* **2012**, *167*, 164.

(29) Rasmussen, A. H.; Strøbæk, D.; Dyhring, T.; Jensen, M. L.; Peters, D.; Grunnet, M.; Timmermann, D. B.; Ahring, P. K. Biophysical and pharmacological characterization of $\alpha6$-containing nicotinic acetylcholine receptors expressed in HEK293 cells. *Brain Res.* **2014**, *1542*, 1.

(30) Xiao, Y.; Meyer, E. L.; Thompson, J. M.; Surin, A.; Wroblewski, J.; Kellar, K. J. Rat $\alpha3/\beta4$ subtype of neuronal nicotinic acetylcholine receptor stably expressed in a transfected cell line: pharmacology of ligand binding and function. *Mol. Pharmacol.* **1998**, *54*, 322.

(31) Xiao, Y.; Baydyuk, M.; Wang, H.; Davis, H. E.; Kellar, K. J. Pharmacology of the agonist binding sites of rat neuronal nicotinic receptor subtypes expressed in HEK 293 cells. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1845.

https://doi.org/10.1021/acs.joc.1c00707
J. Org. Chem. 2021, 86, 8248−8262