Enantiopure Cyclopropane-Bearing Pyridyldiazabicyclo[3.3.0]Octanes As Selective \( \pm 4\beta^2 \)-Nachr Ligands

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Enantiopure Cyclopropane-Bearing PyridylDiazabicyclo[3.3.0]octanes as Selective α4β2-nAChR Ligands

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Supporting Information

ABSTRACT: We report the synthesis and characterization of a series of enantiopure 5-cyclopropane-bearing pyridylDiazabicyclo[3.3.0]octanes that display low nanomolar binding affinities and act as functional agonists at α4β2/nAChR subtype. Structure−activity relationship studies revealed that incorporation of a cyclopropane-containing side chain at the 5-position of the pyridine ring provides ligands with improved subtype selectivity for nAChR β2 subunit-containing nAChR subtypes (β2*-nAChRs) over β4*-nAChRs compared to the parent compound 4. Compound 15 exhibited subnanomolar binding affinity for α4β2- and α4β2*-nAChRs with negligible interaction. Functional assays confirm selectivity for α4β2-nAChRs. Furthermore, using the SmartCube assay system, this ligand showed antidepressant, anxiolytic, and antipsychotic features, while mouse forced-swim assay further confirm the antidepressant-like property of 15.

KEYWORDS: Nicotinic acetylcholine receptor, selective α4β2 partial agonist, N-pyridylDiazabicyclo[3.3.0]octane

Nicotinic acetylcholine receptors (nAChRs) are expressed as pentameric complexes of single (homomeric) or multiple (heteromeric) subunits, which are encoded by 17 different genes (in vertebrates), thus creating a wide variety of nAChR subtypes. The most common nAChR subtypes present in the central nervous system (CNS) are heteropentamers containing α4 and β2 subunits or the homopentamer comprising α7 subunits, while the peripheral nervous system (PNS) consist mainly of α3 subunit combinations (predominantly α3/β4 heteromer).α nAChR subtypes possess unique pharmacological and physiological properties depending on their subunit makeup and identifying ligands that offer selectivity among these subtypes affords opportunities to develop novel therapeutic agents for use in various central nervous system disorders including schizophrenia, depression, Alzheimer’s disease, tobacco addiction, and attention deficit hyperactivity disorder (ADHD).β−δ Moreover, identifying selective ligands would help to attenuate adverse side effects associated with actions at ganglionic α3/β4*-nAChRs (the asterisk indicates that the receptor complex is known to or may contain other subunits than those specified).ε−γ A growing body of evidence indicates that α4β2*-nAChR subtypes appear to play an essential role in depression as well as in cognition, attention, anxiety, and nicotine dependence.β−δ,γ−δ For instance, varenicline, marketed as a smoking cessation pharmacotherapy, is an α4β2-nAChR partial agonist.β,δ Studies have shown that varenicline possesses antidepressant-like effects and also improves cognition in animal models.β,δ However, several side effects such as nausea, mood changes, sleep disturbance, and constipation have been associated with the use of varenicline for smoking cessation, effects that may be related in part to its α3/β4 subtype activity coupled with its 5HT1 activity.β,δ,γ,δ,β Furthermore, efforts have been made to advance the noncompetitive nicotinic antagonist mecamylamine for use in depression; however, this compound failed to show efficacy in human clinical trials.β,δ The development of other nAChR ligands for use in depression thus still represents a therapeutic opportunity.
Sazetidine-A (1), a 3-pyridyl ether possessing an alkynyl substituent at its 5-position, is a highly potent α4/2-nAChR partial agonist (Kᵢ = 0.4 nM) that possesses a 24,000-fold selectivity for α4/2- over α3/4-nAChRs (Figure 1). Sazetidine-A also displays potent anxiolytic, analgesic, and antidepressant features as revealed in studies using animal models.21–23

![Figure 1. Selected nicotinic receptor ligands (1–5).](image)

We recently reported the synthesis and biological characterization of a novel series of α4/2-nAChR partial agonists bearing a cyclopropane ring in place of the acetylenic bond present in Sazetidine-A (1). The cyclopropane ring enforces an orientation of the side chain such that compounds possessing this motif maintained subtype selectivity for α4/2-nAChRs.23 Compounds 2 and 3 are highly selective α4/2-nAChR partial agonists with subnanomolar binding affinities (Kᵢ = 0.1 and 0.2 nM, respectively) and excellent subtype selectivity over α3/4*- and α7-nAChRs.24,25 These compounds also show antidepressant activity in mouse forced swim studies. Compound 4, a 3-pyridyl diazabicyclo[3.3.0]octane, is an α4/2-nAChR agonist having subnanomolar binding affinity (Kᵢ = 0.12 nM for a rat brain α4/2*- subtype) and approximately 400-fold selectivity over α7-nAChRs.

The selectivity of these compounds for α4/2- versus α7-nAChRs can be improved depending on the nature of the R group at position 5 of the pyridine ring, with larger R groups generally showing improved selectivity for α4/2-nAChRs.26 Compound 5, a 3-pyridyl diazabicyclo[3.3.0]octane with a carbamoyl group at position 5, resulted in compounds that generally possessed high binding affinity and selectivity for α4/2- compared to α7-nAChRs.27 In this study, we selected our best ligands23,25 and incorporated the cyclopropane-containing side chain scaffold onto the 5-position of the N-pyridyldiazabicyclo[3.3.0]octane motif 4, in an attempt to improve subtype selectivity toward α4/2*- over ganglionic α3/4*-nAChRs.

The synthetic scheme for the chiral cyclopropylypyridine ligands 9, 10, 13, 15, and 18 is summarized in Scheme 1. The hydroxyl group of the optically pure pyridine intermediate 6 was activated as its triflate 7 and subsequently reacted with tert-butyl cis-hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carboxylate 26, under slightly modified Buchwald–Hartwig conditions to obtain the precursor 8. Deprotection with trifluoroacetic acid (TFA) yielded the secondary amine 9 as its trifluoroacetate salt. The Boc-protected amine 8 was reduced with LiAlH₄ to yield the N-methyl derivative 10.

Next, acylation of 11 using isobutyric anhydride followed by removal of the benzyl group and Buchwald–Hartwig reaction generated 12 after hydrolysis of the isobutyrate protecting group. Cleavage of the Boc group with TFA gave the alcohol 13 as its TFA salt. To generate the fluoride 14, the hydroxyl group of compound 12 was activated as its tosylate and this intermediate treated with n-tetrabutylammonium fluoride to yield the corresponding protected fluoride. Boc deprotection of 14 with TFA afforded the final product 15 as its TFA salt. To prepare the ethyl ether analogue 18, the intermediate 11 was first subjected to the Williamson ether synthesis using ethyl iodide as the alkylating agent. Next, the benzyl group was removed via hydrogenolysis, the amine coupling reaction carried out, and then the Boc group cleaved from 17 to afford 18 as its TFA salt.

In vitro binding affinities (Kᵢ) of all the synthesized 5-cyclopropane-bearing pyridyldiazabicyclo[3.3.0]octanes (9, 10, 13, 15, and 18) were determined using [3H] epibatidine binding competition assays at seven rat nAChR subtypes.28 As illustrated in Table 1, most of these compounds demonstrated relatively high binding affinities for both α4/2- (Kᵢ ranging from 0.4 to 60 nM) and α4/2*-nAChRs (Kᵢ ranging from 1.2 to 120 nM) with poor binding affinities for α3/4-nAChRs (Kᵢ > 2000 nM). This profile suggests a reduced risk of undesirable...
Table 1. Binding Affinities of 11 Ligands at Seven nAChR Subtypes Defined by Competition for \(^{[3]}\text{H}\)Epibatidine Binding

<table>
<thead>
<tr>
<th>compd</th>
<th>(\alpha_2\beta_2)</th>
<th>(\alpha_2\beta_4)</th>
<th>(\alpha_3\beta_2)</th>
<th>(\alpha_3\beta_4)</th>
<th>(\alpha_4\beta_2)</th>
<th>(\alpha_4\beta_2^{*})</th>
<th>(\alpha_4\beta_4)</th>
<th>selectivity ((\alpha_3\beta_4/\alpha_4\beta_2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1^a)</td>
<td>10^4</td>
<td>0.40</td>
<td>0.90</td>
<td>24000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2^a)</td>
<td>&gt;10^4</td>
<td>0.10</td>
<td>0.30</td>
<td>&gt;100000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3^a)</td>
<td>3200</td>
<td>0.20</td>
<td>0.90</td>
<td>16000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>46</td>
<td>2.7</td>
<td>200</td>
<td>0.50</td>
<td>1.5</td>
<td>27</td>
<td>400</td>
</tr>
<tr>
<td>9</td>
<td>0.30 ± 0.10</td>
<td>380</td>
<td>26 ± 7.0</td>
<td>&gt;10^4</td>
<td>4.1 ± 2.0</td>
<td>1.2 ± 0.2</td>
<td>170</td>
<td>&gt;2400</td>
</tr>
<tr>
<td>10</td>
<td>25 ± 3.0</td>
<td>&gt;10^4</td>
<td>&gt;10^4</td>
<td>2500</td>
<td>58 ± 18</td>
<td>120</td>
<td>1400</td>
<td>40</td>
</tr>
<tr>
<td>13</td>
<td>0.60 ± 0.10</td>
<td>260</td>
<td>19 ± 5.0</td>
<td>7900</td>
<td>3.1 ± 1.0</td>
<td>2.4 ± 0.4</td>
<td>110</td>
<td>2500</td>
</tr>
<tr>
<td>15</td>
<td>0.40</td>
<td>65</td>
<td>10</td>
<td>2500</td>
<td>1.6</td>
<td>3.6</td>
<td>37</td>
<td>1600</td>
</tr>
<tr>
<td>18</td>
<td>0.20</td>
<td>390</td>
<td>6.8</td>
<td>&gt;10^4</td>
<td>0.40</td>
<td>1.9</td>
<td>160</td>
<td>&gt;25000</td>
</tr>
<tr>
<td>nicotine</td>
<td>5.5</td>
<td>70</td>
<td>29</td>
<td>260</td>
<td>4.9</td>
<td>9.8</td>
<td>23</td>
<td>93</td>
</tr>
<tr>
<td>varenicline</td>
<td>8.6</td>
<td>0.40</td>
<td>110</td>
<td>210</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)See Supporting Information. \(^b\)\(\alpha_4\beta_2^{*}\), endogenous receptors prepared from rat forebrain. Besides \(\alpha_4\) and \(\beta_2\), other unidentified subunits may also be present. \(\text{K}_i\) values for nicotine were taken from the PDSP Assay Protocol Book (http://pdsp.med.unc.edu/). \(^c\)NA: not active, defined as <50% inhibition of binding in the primary assay at 10 \(\mu\)M. \(\text{K}_i\) values for varenicline are from the literature. \(\text{K}_i\) values for 2 are from the literature.  \(^d\)Values for 2 are from the literature.

Table 2. Functional Potencies and Efficacies of Ligands: Agonism and inactivation of Human \(\alpha_4\beta_2\)-nAChRs.

<table>
<thead>
<tr>
<th>compd</th>
<th>(EC_{50}) (nM)</th>
<th>pEC_{10}</th>
<th>HS-(\alpha_4\beta_2) efficacy (%)</th>
<th>LS-(\alpha_4\beta_2) efficacy (%)</th>
<th>IC_{50} (nM)</th>
<th>pIC_{10}</th>
<th>efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1^a)</td>
<td>5.8</td>
<td>100</td>
<td>76 ± 14</td>
<td>0.40 ± 5.1</td>
<td>4.8</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>(2^a)</td>
<td>18</td>
<td>60</td>
<td>ND</td>
<td>ND</td>
<td>5.6</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>7.9 ± 0.10</td>
<td>76 ± 14</td>
<td>0.40 ± 5.1</td>
<td>11</td>
<td>8.0 ± 0.04</td>
<td>77 ± 1.0</td>
</tr>
<tr>
<td>10</td>
<td>&gt;1000</td>
<td>&lt;6.0</td>
<td>ND</td>
<td>ND</td>
<td>&gt;10^3</td>
<td>&lt;6.0</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>7.8 ± 0.10</td>
<td>100 ± 7.0</td>
<td>1.7 ± 4.0</td>
<td>15</td>
<td>7.8 ± 0.04</td>
<td>73 ± 2.0</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>7.6 ± 0.10</td>
<td>110 ± 7.0</td>
<td>-9.4 ± 4.0</td>
<td>20</td>
<td>7.7 ± 0.04</td>
<td>74 ± 2.0</td>
</tr>
<tr>
<td>18</td>
<td>20</td>
<td>7.7 ± 0.10</td>
<td>88 ± 4.0</td>
<td>-7.0 ± 4.0</td>
<td>12</td>
<td>7.9 ± 0.10</td>
<td>76 ± 4.0</td>
</tr>
<tr>
<td>nicotine</td>
<td>300</td>
<td>6.5 ± 0.10</td>
<td>120 ± 9.0</td>
<td>70 ± 6.0</td>
<td>430</td>
<td>6.4 ± 0.10</td>
<td>92 ± 2.0</td>
</tr>
</tbody>
</table>

\(^a\)See Supporting Information for details. The term “inactivation” is used because compounds may be acting as desensitizing receptors or as competitive or noncompetitive antagonists, and further work is needed to make such a distinction. Potencies (EC_{50} or IC_{50} values) and efficacies were measured for actions at a mixture of high-sensitivity (HS) and low-sensitivity (LS) \(\alpha_4\beta_2\)-nAChR. Reported errors are the standard error of the mean (SEM) for all values. \(^b\)Results for compound 1 were obtained from ref 18. Results for compound 2 were obtained from ref 20. \(^d\)ND: Not determined. The efficacy was not determined if the EC_{50} or the IC_{50} value was greater than 1000 nM.

Side effects associated with binding to ganglionic \(\alpha_3\beta_4\)-nAChRs. These compounds also showed good selectivity for \(\beta_2^{*}\)-nAChRs (\(\alpha_2\beta_2\), \(\alpha_3\beta_2\), \(\alpha_4\beta_2\), and \(\alpha_4\beta_2^{*}\)-nAChRs) over \(\beta_4^{*}\)-nAChRs (\(\alpha_2\beta_4\), \(\alpha_3\beta_4\), and \(\alpha_4\beta_4\)-nAChRs) compared to nicotine. The N-methyl-bearing analogue 10 showed decreased binding affinity for \(\alpha_4\beta_2\)-nAChRs (\(K_i = 58\) nM) compared to the corresponding unsubstituted compound 9 (\(K_i = 4.1\) nM), resulting in a 60-fold reduction in selectivity for \(\alpha_4\beta_2\) over \(\alpha_3\beta_4\)-nAChRs. The binding affinity of ligand 13, bearing a terminal hydroxyl group, at \(\alpha_4\beta_2\)-nAChRs was similar to its fluoro-containing counterpart 15. However, alcohol 13 has an improved selectivity ratio (\(\alpha_3\beta_4/\alpha_4\beta_2\)) compared to 15. Of the new analogues made and tested herein, the ethoxy derivative 18 displayed the best binding affinity for \(\alpha_4\beta_2\)-nAChRs (\(K_i = 0.40\) nM), and it was found to be inactive at \(\alpha_3\beta_4\)-nAChRs (\(K_i = >10000\) nM). Selected ligands were also tested at \(\alpha_7^{*}\) and \(\alpha_7^{*}\)-nAChRs, (\(\alpha_7^{*}\), endogenous receptors prepared from rat forebrain) and they were found to be devoid of activity at the highest concentration used (10 \(\mu\)M), with the exception of 18 (\(K_i = 680\) nM at \(\alpha_7\)-nAChRs) (data not shown).

Functional activity of all compounds was characterized at human \(\alpha_4\beta_2\), \(\alpha_3\beta_4^{*}\), and \(\alpha_1\beta_1\gamma_6\)-nAChRs using SH-EP1-h\(\alpha_4\beta_2\), SH-SYSY, and TE671/RD cells, respectively, and \(\text{86Rb}^+\) ion efflux assays. Note that \(\alpha_4\beta_2\)-nAChRs actually exist as two isoforms differing in sensitivity to nicotine or acetylcholine: high sensitivity (HS) (\(\alpha_4\beta_2\)\(\beta_2\))-nAChRs and low sensitivity (LS) (\(\alpha_4\beta_2\)\(\beta_2\))-nAChRs. Sazetidine-A is unusual in that it is a fully efficacious agonist at HS (\(\alpha_4\beta_2\)\(\beta_2\))-nAChRs, but it has much weaker efficacy at the LS isoform relative to conventional agonists like ACh and nicotine. This is because sazetidine-A only activates the HS phase of LS receptor function but does not activate the LS phase due to its lack of activity at the \(\alpha_4/\alpha_4\) subunit interface present in the LS isoform. 30 Efficacy of ligands at HS vs LS \(\alpha_4\beta_2\)-nAChR can be assayed by reference to proportions of those isoforms expressed in a given preparation of cells being studied as defined by function elicited by sazetidine-A. As seen in Table 2, the analogues tested share sazetidine-A’s characteristic discrimination between HS- and LS-\(\alpha_4\beta_2\) nAChR isoforms. Tested compounds had agonist activity at \(\alpha_4\beta_2\)-nAChRs with EC_{50} < 30 nM, with the exception of 10, which showed no activity (Table 2). For the
ligands evaluated, there was neither agonist nor antagonist activity at ganglionic \( \alpha 3/\beta 4 \)- or muscle-type \( \alpha 1/\beta 1 \gamma 8 \)-nAChRs or the potency was too low to characterize without testing at concentrations above 10 \( \mu \)M. The methoxy analogue 9 showed similar \( EC_{50} \) and \( IC_{50} \) values (14 and 11 nM) to those for the azetidine-containing ligand 2 (\( EC_{50} = 18 \) nM and \( IC_{50} = 5.6 \) nM, Table 2). Interestingly, the hydroxyl (13) and fluoro (15) analogues showed the highest efficacies at 100% and 110%, respectively, for stimulation of HS \( (\alpha 4)_{2}(\beta 2)_{2} \)-nAChRs.

Compounds 13 and 15, however, had functional inactivation efficacies similar to those of other compounds tested in this study (Table 2). The ethoxy analogue 18, which had the best binding affinity to \( \alpha 4/\beta 2 \)-nAChRs (\( K_i = 0.40 \) nM) among the compounds tested here, also showed excellent activity at \( \alpha 4/\beta 2 \)-nAChRs in functional agonism and inactivation assays (\( EC_{50} = 20 \) nM and \( IC_{50} = 12 \) nM). Of note, none of the tested ligands appear to have any significant intrinsic activity at LS \( (\alpha 4)_{2}(\beta 2)_{2} \) and have apparent efficacies ranging from 76% to 110% at HS \( (\alpha 4)_{2}(\beta 2)_{2} \)-nAChRs.

Preliminary in vivo evaluation of the nicotinic ligands for behavioral effects was carried out using SmartCube, an automated system that analyzes the behaviors of compound-treated mice captured on digital video with the aid of computer algorithms.\(^{31} \) The behavioral signature of a test compound is compared with a database of behavioral signatures obtained from a large set of diverse reference compounds. Thus, we are able to make predictions as to the possible neuropharmacological activity of a test compound relative to major classes of compounds such as anxiolytics, antipsychotics, and antidepressants. All compounds were administered at doses of 5 or 10 mg/kg. Compounds 9, 13, 15, and 18 were found to produce behavioral signatures that have features of antidepressants, anxiolytics, and antipsychotics with little or no side effect profiles (Figure 2). Consistent with its lower potency in the radioligand binding and functional studies, compound 10 is relatively inactive in SmartCube and does not show the behavioral signature of compounds 9, 13, 15, and 18.

Next, to further establish the ability of these compounds to penetrate the blood–brain barrier, compound 15 was selected for mouse in vivo pharmacokinetic (PK) studies. The plasma and brain concentrations of compound 15 in male CD-1 mice after a single intraperitoneal (IP) injection at a dose of 10 mg/kg were measured. The concentration of 15 reached a value of 197 and 256 ng/g at 30 and 120 min in the brain and 828 and 256 ng/mL at 30 and 120 min in plasma (Table 3). The brain to plasma ratio of compound 15 was found to be 0.24 at 30 min and 1.75 at 120 min, indicating acceptable CNS penetration.

Furthermore, the binding of 15 to protein in male CD-1 mouse plasma and brain tissue was determined using equilibrium dialysis. Binding of 15 was evaluated at a final concentration of 1 \( \mu \)M. The percentage of binding of compound 15 in mouse plasma and brain tissue was 27% and 73%, respectively, after a 6 h incubation period. These results thus indicate that sufficient amounts of the unbound drug are available in the brain to exert a pharmacological action.

On the basis of the SmartCube data and brain concentration levels of compound 15, we decided to further probe the possible antidepressant action of compound 15. We thus examined the effects of compound 15 in the classical mouse forced-swim test,\(^{32} \) an assay in which mice are placed into a beaker of water, and the time spent passively floating in the water (immobility) is recorded (Figure 3). Most traditional antidepressants decrease the amount of time the mouse spends immobile. Mice were administered compound 15 (30 mg/kg of the free base) 15 min prior to testing or the selective serotonin reuptake inhibitor sertraline, as a positive control (20 mg/kg). Compound 15 exhibited an antidepressant-like effect when administered IP with a significant reduction in immobility at a single dose as displayed in the bar graph in Figure 3.
In summary, we describe the synthesis, pharmacological evaluation, and behavioral characterization of some 5-cyclopropane-bearing pyridydiazabicyclo[3.3.0]octanes as nAChR ligands. All tested ligands with the exception of compound 10 showed excellent binding affinities for both α4β2- and α4β2*- nAChRs from the rat (Kᵢ values ranging from 0.4 to 4.1 nM) and poor affinity for rat α3β4-nAChRs (Kᵢ >2400 nM). In functional studies, these ligands acted as potent agonists at human α4β2-nAChRs and were inactive at both ganglionic α3β4*- or muscle-type α1β1δ- nAChRs. In this series, the fluoro-analogue 15 was found to possess subnanomolar binding affinity, a 1550-fold selectivity for α4β2- versus α3β4-nAChRs, as well as good agonist efficacy in the functional studies. Compound 15 achieves a brain concentration of ~0.70 μM at 30 min, and this is over 400-times more than its binding affinity at the α4β2-nAChR. Compound 15 was found to display antidepressant-like properties in the mouse forced-swim test. The above data support our hypothesis that the incorporation of the cyclopropane side chain at the 5-position of the cyclopropane side chain at the 5-position of the pyridydiazabicyclo[3.3.0]octanes as nAChR ligands would improve subtype selectivity for α4β2- over α3β4-nAChRs when compared to the parent compound 4, thus implying that the nature of the substitution at the position 5 plays a vital role in attenuating possible side effects associated with ganglionic α3β4*-nAChRs. These potent and selective nAChR ligands produced antidepressant/anxiolytic-like properties in the SmartCube test, and thus, they may serve as chemical probes in further exploring various aspects of nicotinic receptor function related to mood disorders.

**ASSOCIATED CONTENT**

Supporting Information
Experimental procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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**ABBREVIATIONS**

nAChR, nicotinic acetylcholine receptor; ADHD, attention deficit hyperactivity disorder; TFA, trifluoroacetic acid; NIMH-PDSP, National Institute of Mental Health Psychoactive Drug Screening Program; HS, high sensitivity; LS, low sensitivity; IP, intraperitoneal

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