Aza-tryptamine substrates in monoterpene indole alkaloid biosynthesis

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2. Supplemental Experimental Procedures
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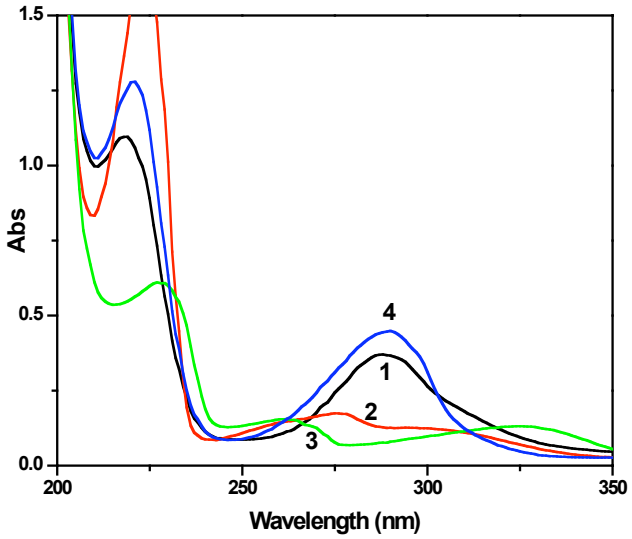
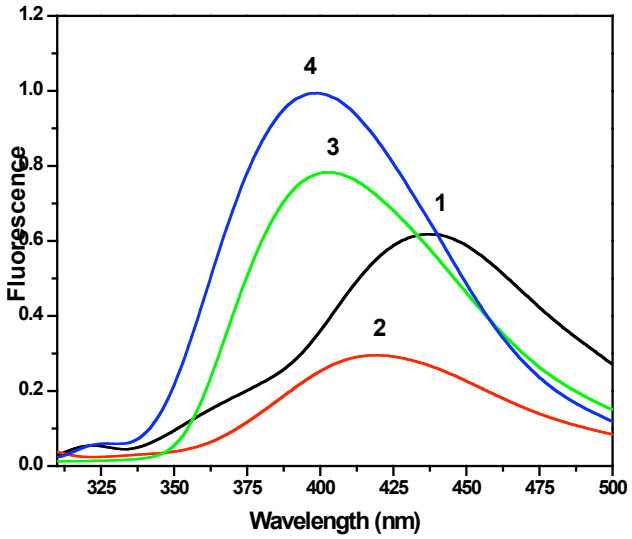
3. Supplemental References

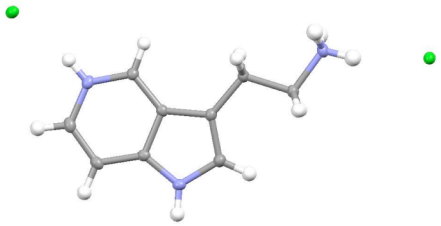
1. Supplemental Data



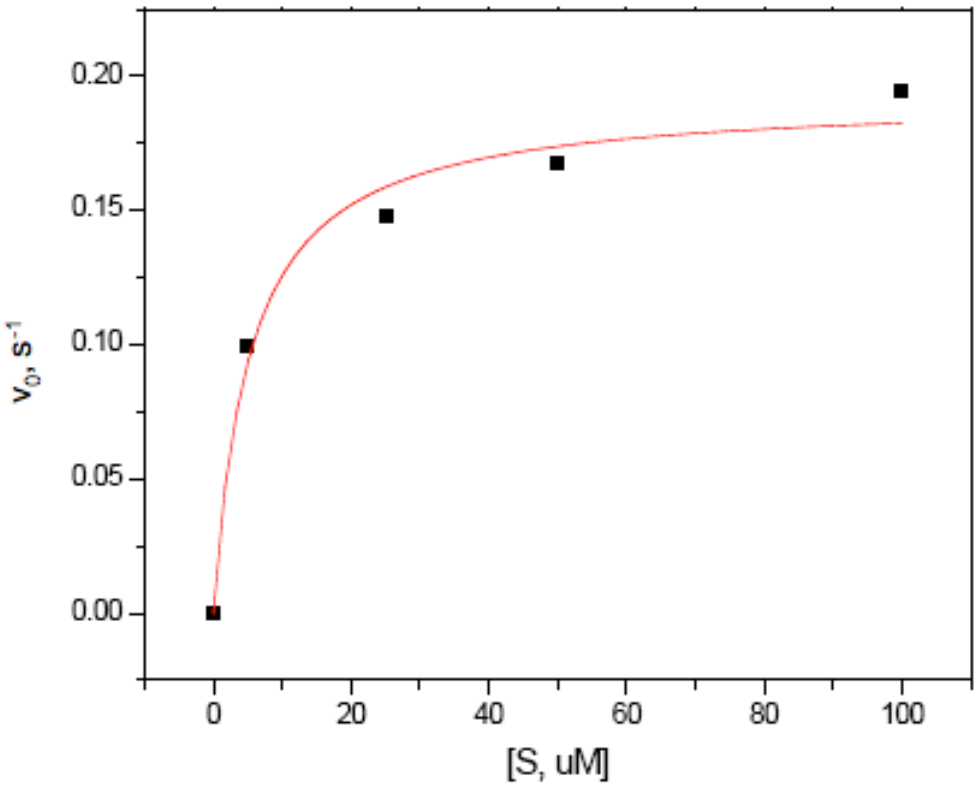
Supplementary Figure 1. UV spectra of 4-aza-tryptamine **1**, 5-aza-tryptamine **2**, 6-aza-tryptamine **3** and 7-aza-tryptamine **4**. All UV spectra of aza-tryptamines **1**-**4** were obtained on a Cary 50 Bio (Varian) UV/Vis spectrometer using sample concentrations of 50 μM in PBS (50 mM, pH 7.0). Fluorescence spectra were conducted on a Horiba Jobin Fluoromax-3 in a 1 cm path length quartz cell.



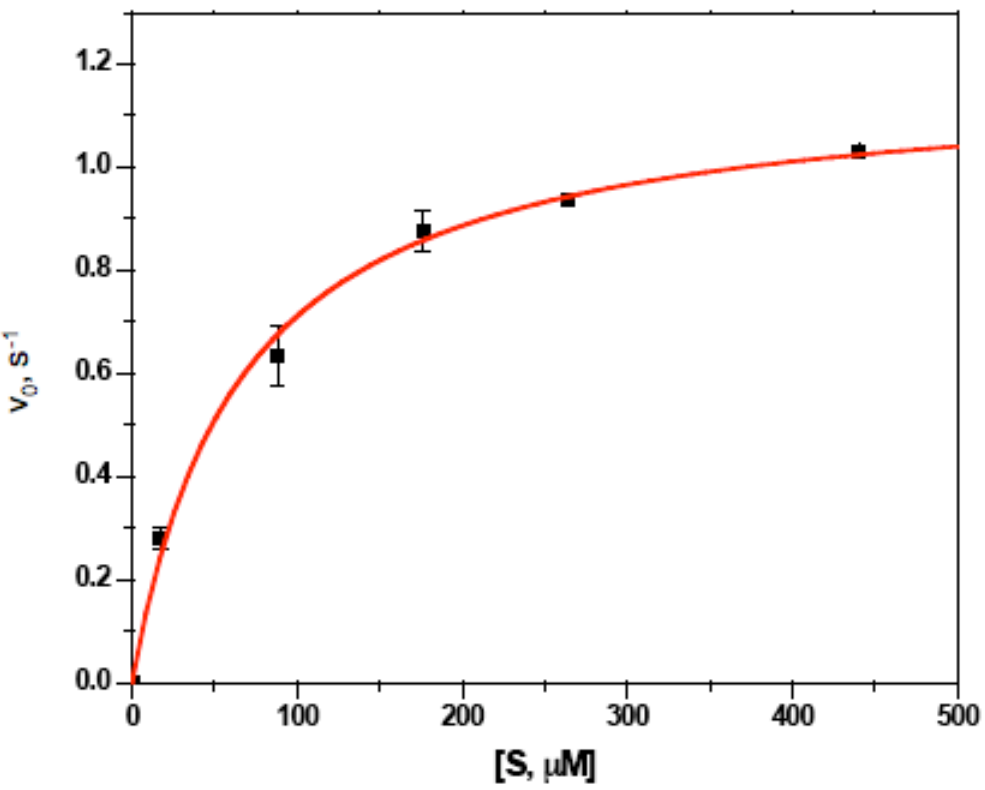
Supplementary Figure 2. Fluoresence spectra of 4-aza-tryptamine **1**, 5-aza-tryptamine **2**, 6-aza-tryptamine **3** and 7-aza-tryptamine **4**. All fluorescence spectra of **1**-**4** (5 μM in PBS, 50 mM, pH 7.0) were recorded at the following maximum excitation and emission wavelengths: **1** 288 nm excitation, 437 nm emission; **2** 275 nm excitation, 419 nm emission; **3** 262 nm excitation, 404 nm emission; **4** 290 nm excitation, 399 nm emission.



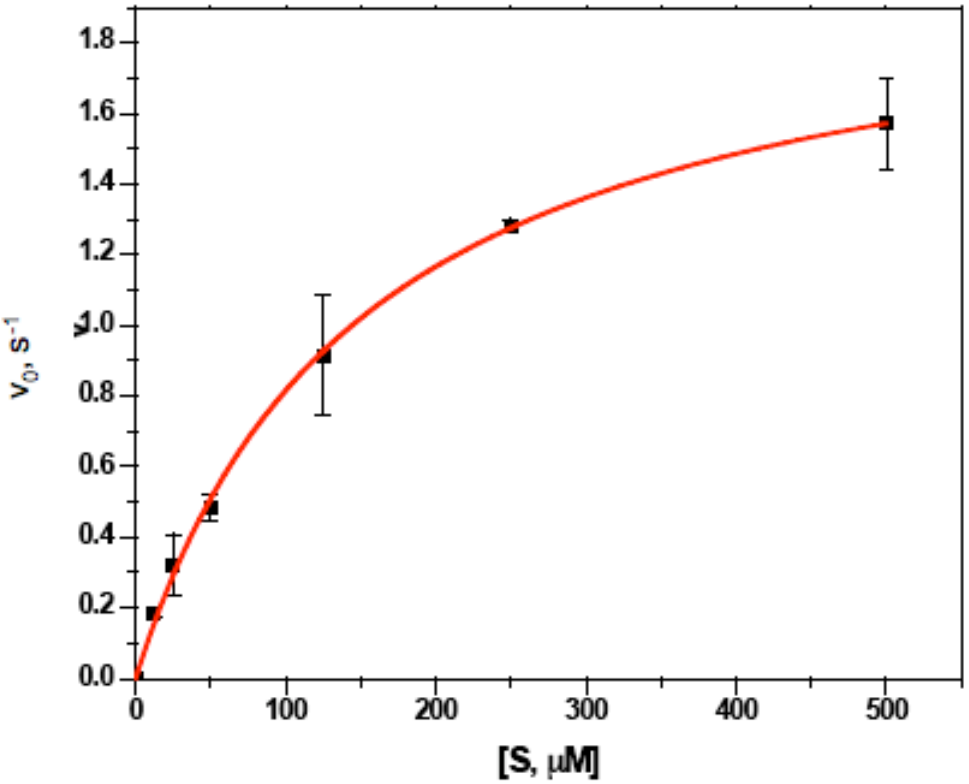
Supplementary Figure 3. X-ray crystal structure of 5-azaindole tryptamine hydrochloride **2**. Coordinates are listed at the end of the Supplementary Data section.



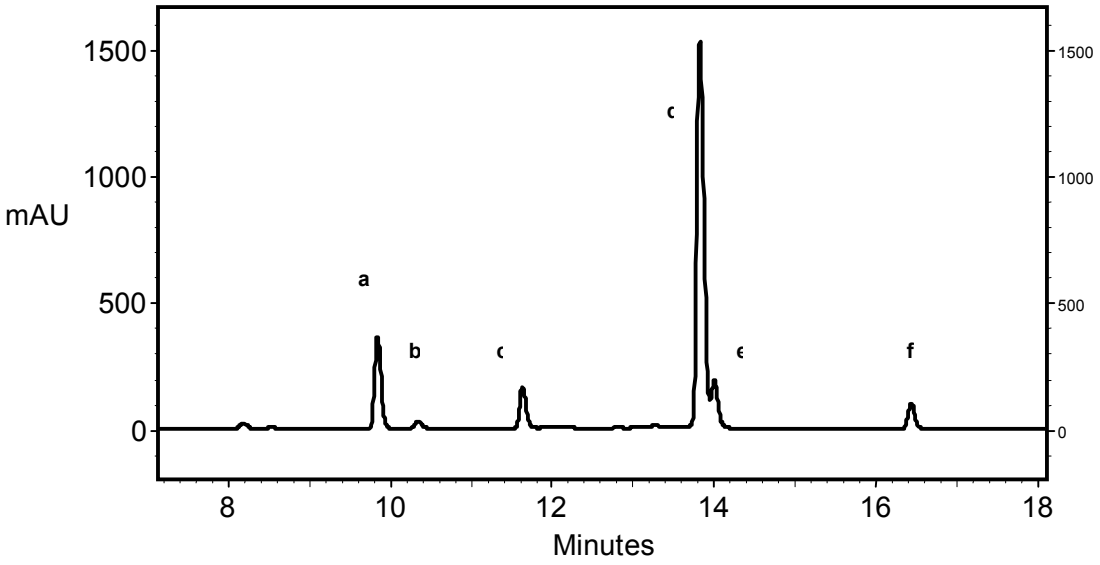
Supplementary Figure 4. Kinetic data for tryptamine **5** and strictosidine synthase (*C. roseus*). Initial rates were normalized by enzyme concentration ($[E]_o$ = 2 nM).



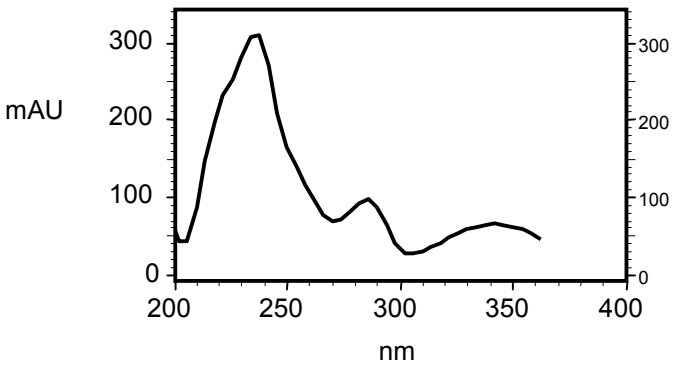
Supplementary Figure 5. Kinetic data for 4-aza-tryptamine **1** and strictosidine synthase (*C. roseus*). Initial rates were normalized by enzyme concentration ($[E]_o$= 0.2 μM).



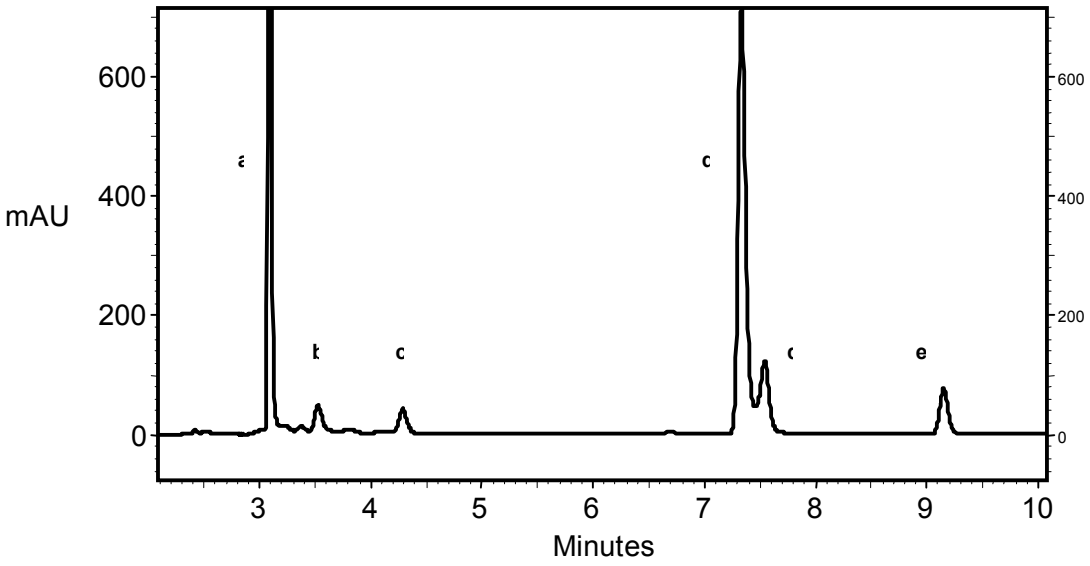
Supplementary Figure 6. Kinetic data for 7-aza-tryptamine **4** and strictosidine synthase (*C. roseus*). Initial rates were normalized by enzyme concentration ($[E]_o$= 0.2 μM).



Supplementary Figure 7. HPLC chromatogram showing deglucosylation of azastrictosidine **7** derived from **1**. The enzymatic reaction containing strictosidine synthase and strictosidine glucosidase was directly injected into analytical HPLC after 24 hours at 30˚C to monitor reaction progress. 4-Aza-tryptamine **1** (500 µM), secologanin **6** (2.5 mM), strictosidine synthase, strictosidine glucosidase and internal standard (naphthalene acetic acid) (60 µM) in PBS buffer (50mM, pH 7.0).
a, **1** (9.8 min); b, secologanin **6** (10.4 min); c, azastrictosidine **7** (11.6 min); d, e, deglucosylated strictosidine (13.8 min, 14.0 min); f, internal standard (16.4 min). Gradient, 0-70% acetonitrile in water with 0.1% trifluoroacetic acid over 20min, monitoring at 290nm.

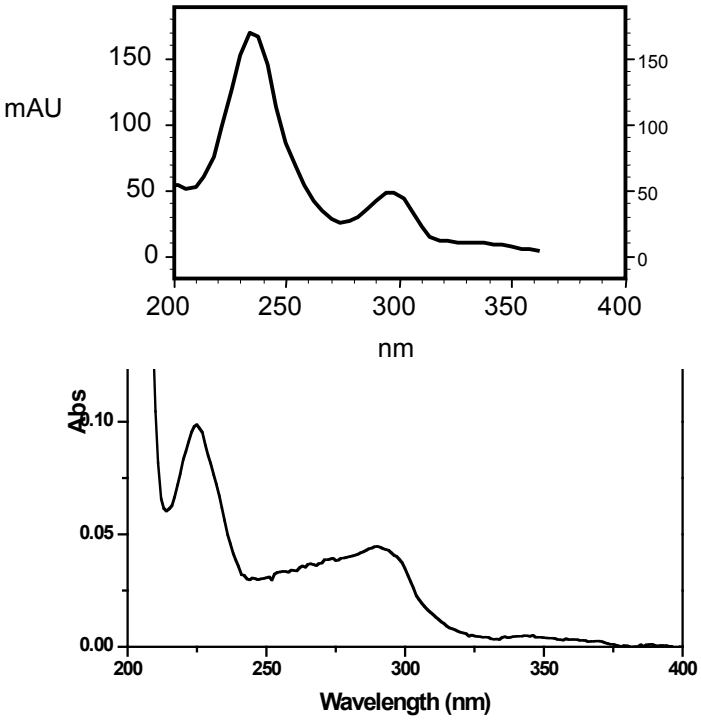


Supplementary Figure 8. UV spectrum of 4-aza-strictosidine **7.**



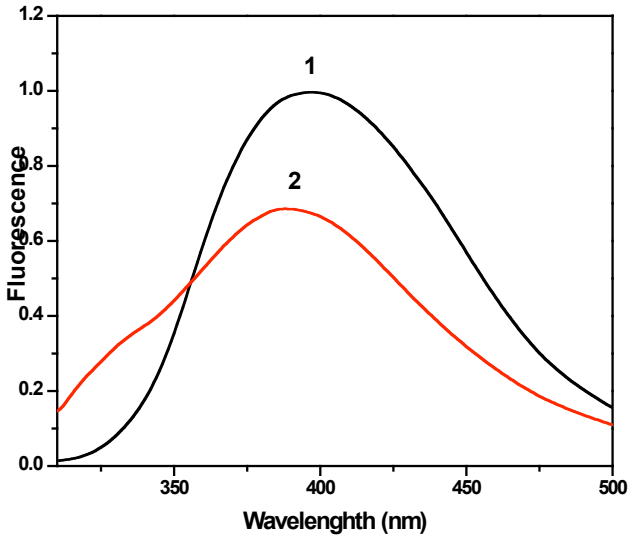
Supplementary Figure 9. HPLC chromatogram showing deglucosylation of azastrictosidine **10** derived from **4**. The enzymatic reaction containing strictosidine synthase and strictosidine glucosidase was directly injected into analytical HPLC after 24 hours at 30˚C to monitor reaction progress. 7-Aza-tryptamine **4** (500 µM),

secologanin **6** (2.5 mM), strictosidine synthase, strictosidine glucosidase and internal standard (naphthalene acetic acid) (60 μM) in PBS buffer (50mM, pH 7.0). a, **4** (3.1 min); b, secologanin **6** (3.5 min); c, azastrictosidine **10** (4.3 min); d, e, deglucosylated strictosidine (7.3 min, 7.5 min); f, internal standard (9.1 min). Gradient, 5-70% acetonitrile in water with 0.1% trifluoroacetic acid over 15 minutes, monitoring at 290nm.

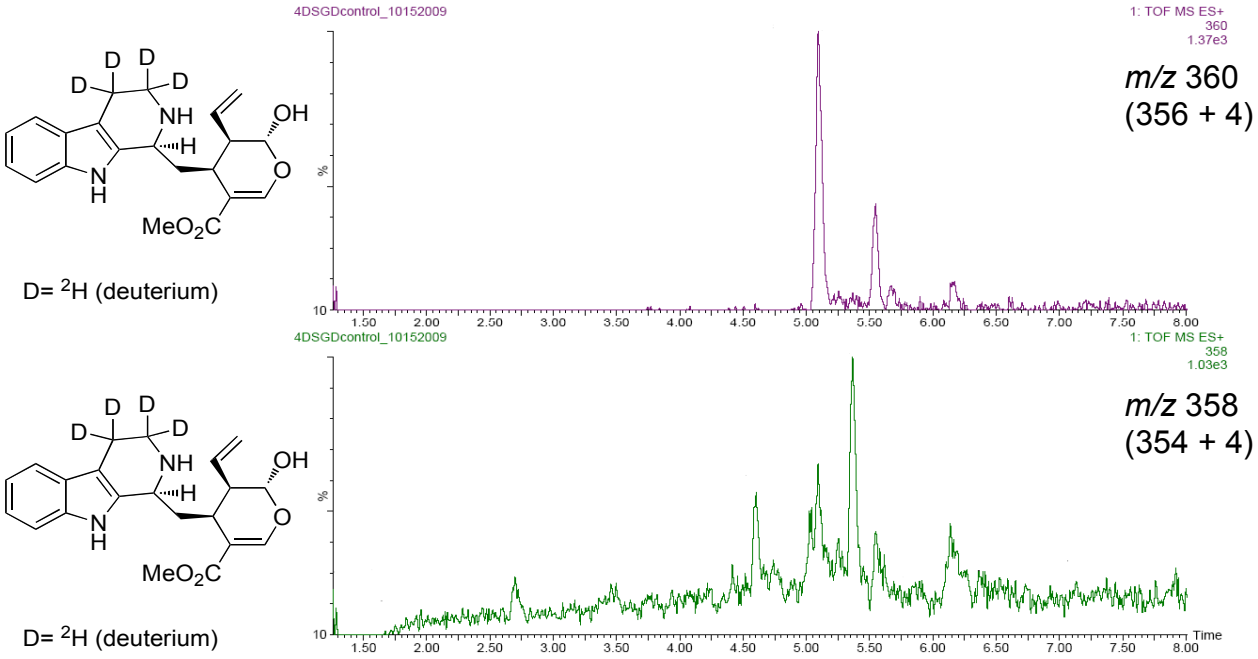


rictosidine **10**.

Supplementary Figure 11. UV spectrum of 7-azaindole isositsirikine **14** (λ_{Max}(n-π^*) at 290nm).



Supplementary Figure 12. Fluorescence spectra of **14**. Trace 1 = **4**; Trace 2 = **14**.



Supplementary Figure 13. *C. roseus* cell cultures incubated with "natural" deglycosylated strictosidine **11** (derived from tryptamine **5**). This control experiment

ensured that none of the peaks marked with an asterisk in Figure 4 derived from deglycosylated strictosidine. Culture conditions, substrate concentration and length of sub-culture were identical to those described in the manuscript text with the aza-analogs. Isotopically labeled strictosidine (deuterium-4) was used to facilitate product identification. Therefore, we examined peaks at *m/z* 358 (354+4) and *m/z* 360 (356+4). No compounds with a retention time between 1.5 and 4.5 minutes were observed. Control experiments with cultures not supplemented with any substrate also lack compounds in this retention time range as shown in Figure 4. We therefore conclude that the compounds marked with an asterisk in Figure 4 are formed only when the aza-tryptamine analog is present.

Table S1-S6. X-ray data for 5-aza-tryptamine 2.

Table 1. Crystal data and structure refinement for 09052.

Identification code	09052	
Empirical formula	C9 H13 Cl2 N3	
Formula weight	234.12	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P2(1)/c	
Unit cell dimensions	a = 9.1737(8) Å	α= 90°.
	b = 14.0341(12) Å	β= 110.5000(10)°.
	c = 9.0956(8) Å	γ = 90°.
Volume	1096.85(17) $Å^3$	
Z	4	
Density (calculated)	1.418 Mg/m^3	
Absorption coefficient	0.557 mm^{-1}	
F(000)	488	
Crystal size	0.40 x 0.30 x 0.25 mm^3	

Theta range for data collection	2.37 to 29.57°.
Index ranges	-12<=h<=12, -19<=k<=19, -12<=l<=12
Reflections collected	19729
Independent reflections	3080 [R(int) = 0.0328]
Completeness to theta = 29.57°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.8734 and 0.8080
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3080 / 5 / 142
Goodness-of-fit on F^2	1.059
Final R indices [I>2sigma(I)]	R1 = 0.0261, wR2 = 0.0700
R indices (all data)	R1 = 0.0291, wR2 = 0.0725
Largest diff. peak and hole	0.374 and -0.314 e.$Å^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 09052. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	6273(1)	1627(1)	8637(1)	17(1)
N(2)	8892(1)	545(1)	2497(1)	16(1)
N(3)	7335(1)	-1420(1)	4830(1)	17(1)
C(1)	6356(1)	732(1)	7786(1)	16(1)
C(2)	7057(1)	958(1)	6536(1)	15(1)
C(3)	7282(1)	90(1)	5684(1)	14(1)
C(4)	7879(1)	92(1)	4418(1)	13(1)
C(5)	8423(1)	796(1)	3683(1)	14(1)
C(6)	8858(1)	-365(1)	1969(1)	18(1)
C(7)	8358(1)	-1103(1)	2655(1)	17(1)
C(8)	7875(1)	-870(1)	3910(1)	14(1)
C(9)	6968(1)	-844(1)	5893(1)	16(1)
Cl(1)	4755(1)	1684(1)	11258(1)	16(1)
Cl(2)	9837(1)	1905(1)	367(1)	17(1)

Table 3. Bond lengths [Å] and angles [°] for 09052.

N(1)-C(1)	1.4917(14)
N(1)-H(1C)	0.923(13)
N(1)-H(1D)	0.907(13)
N(1)-H(1E)	0.915(13)
N(2)-C(5)	1.3409(14)
N(2)-C(6)	1.3602(15)
N(2)-H(2)	0.864(12)
N(3)-C(8)	1.3529(14)
N(3)-C(9)	1.3891(14)
N(3)-H(3)	0.866(12)
C(1)-C(2)	1.5228(15)
C(1)-H(1A)	0.9900
C(1)-H(1B)	0.9900
C(2)-C(3)	1.4977(14)
C(2)-H(2A)	0.9900
C(2)-H(2B)	0.9900
C(3)-C(9)	1.3693(15)
C(3)-C(4)	1.4372(14)
C(4)-C(5)	1.3808(14)
C(4)-C(8)	1.4262(14)
C(5)-H(5)	0.9500
C(6)-C(7)	1.3691(16)
C(6)-H(6)	0.9500
C(7)-C(8)	1.4005(15)
C(7)-H(7)	0.9500
C(9)-H(9)	0.9500

C(1)-N(1)-H(1C)	107.1(10)
C(1)-N(1)-H(1D)	110.1(10)
H(1C)-N(1)-H(1D)	111.3(14)
C(1)-N(1)-H(1E)	112.1(10)
H(1C)-N(1)-H(1E)	109.7(14)
H(1D)-N(1)-H(1E)	106.7(14)
C(5)-N(2)-C(6)	123.79(10)
C(5)-N(2)-H(2)	119.9(10)

C(6)-N(2)-H(2)	116.3(10)
C(8)-N(3)-C(9)	109.24(9)
C(8)-N(3)-H(3)	125.7(10)
C(9)-N(3)-H(3)	124.6(10)
N(1)-C(1)-C(2)	108.89(9)
N(1)-C(1)-H(1A)	109.9
C(2)-C(1)-H(1A)	109.9
N(1)-C(1)-H(1B)	109.9
C(2)-C(1)-H(1B)	109.9
H(1A)-C(1)-H(1B)	108.3
C(3)-C(2)-C(1)	112.81(9)
C(3)-C(2)-H(2A)	109.0
C(1)-C(2)-H(2A)	109.0
C(3)-C(2)-H(2B)	109.0
C(1)-C(2)-H(2B)	109.0
H(2A)-C(2)-H(2B)	107.8
C(9)-C(3)-C(4)	105.74(9)
C(9)-C(3)-C(2)	129.28(10)
C(4)-C(3)-C(2)	124.97(9)
C(5)-C(4)-C(8)	118.72(9)
C(5)-C(4)-C(3)	133.87(10)
C(8)-C(4)-C(3)	107.40(9)
N(2)-C(5)-C(4)	118.30(10)
N(2)-C(5)-H(5)	120.8
C(4)-C(5)-H(5)	120.8
N(2)-C(6)-C(7)	121.31(10)
N(2)-C(6)-H(6)	119.3
C(7)-C(6)-H(6)	119.3
C(6)-C(7)-C(8)	116.53(10)
C(6)-C(7)-H(7)	121.7
C(8)-C(7)-H(7)	121.7
N(3)-C(8)-C(7)	131.27(10)
N(3)-C(8)-C(4)	107.41(9)
C(7)-C(8)-C(4)	121.32(10)
C(3)-C(9)-N(3)	110.20(10)
C(3)-C(9)-H(9)	124.9

N(3)-C(9)-H(9)	124.9

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 09052. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	19(1)	18(1)	17(1)	-1(1)	9(1)	2(1)
N(2)	15(1)	18(1)	16(1)	4(1)	7(1)	2(1)
N(3)	19(1)	13(1)	19(1)	0(1)	7(1)	-1(1)
C(1)	17(1)	16(1)	17(1)	-1(1)	8(1)	0(1)
C(2)	16(1)	15(1)	16(1)	0(1)	8(1)	0(1)
C(3)	12(1)	15(1)	15(1)	0(1)	5(1)	1(1)
C(4)	11(1)	14(1)	14(1)	-1(1)	4(1)	1(1)
C(5)	13(1)	14(1)	16(1)	1(1)	5(1)	2(1)
C(6)	17(1)	22(1)	15(1)	1(1)	6(1)	5(1)
C(7)	18(1)	16(1)	16(1)	-2(1)	4(1)	3(1)
C(8)	13(1)	13(1)	15(1)	0(1)	3(1)	1(1)
C(9)	16(1)	17(1)	17(1)	0(1)	7(1)	-1(1)
Cl(1)	19(1)	14(1)	18(1)	1(1)	10(1)	1(1)
Cl(2)	19(1)	16(1)	17(1)	0(1)	7(1)	-3(1)

Table 5. Hydrogen coordinates ($x\ 10^4$) and isotropic displacement parameters ($Å^2 x\ 10^{\ 3}$) for 09052.

	x	y	z	U(eq)
H(1C)	7284(16)	1814(11)	9183(18)	26
H(1D)	5749(17)	2082(11)	7946(17)	26
H(1E)	5751(18)	1542(11)	9320(17)	26
H(2)	9234(16)	974(10)	2018(16)	19
H(3)	7129(17)	-2023(9)	4701(17)	20
H(1A)	5300	462	7289	19

H(1B)	7008	256	8532	19
H(2A)	6366	1410	5769	18
H(2B)	8075	1275	7039	18
H(5)	8464	1441	4009	17
H(6)	9188	-490	1108	21
H(7)	8340	-1741	2298	20
H(9)	6557	-1066	6655	20

Table 6. Hydrogen bonds for 09052 [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(1)-H(1C)...Cl(2)#1	0.923(13)	2.213(14)	3.1170(11)	166.1(14)
N(1)-H(1D)...Cl(1)#2	0.907(13)	2.282(13)	3.1827(10)	172.2(14)
N(1)-H(1E)...Cl(1)	0.915(13)	2.261(13)	3.1550(10)	165.5(14)
N(2)-H(2)...Cl(2)	0.864(12)	2.202(13)	3.0537(10)	168.4(13)
N(3)-H(3)...Cl(1)#3	0.866(12)	2.447(13)	3.2263(10)	150.0(13)

Symmetry transformations used to generate equivalent atoms:

#1 x,y,z+1 #2 x,-y+1/2,z-1/2 #3 -x+1,y-1/2,-z+3/2

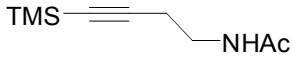
2. Supplemental Experimental Procedures

Materials

C18 cartridges were purchased from Honeywell Burdick & Jackson. The compound 4-trimethylsilyl-3-butyn-1-ol was purchased from Wako, and 2-amino-3-iodopyridine, 3-amino-4-iodopyridine, 3-amino-2-bromopyridine and 4-amino-3-iodopyridine were purchased from Alfa Aesar. Tryptamine, $Pd(dppf)Cl_2.CH_2Cl_2$ and trans-*N*,*N*'-dimethylcyclohexane were purchased from Aldrich. All reagents were used without further purification.

Synthetic procedures

N-(4-trimethylsiliylbut-3-ynyl)acetamide



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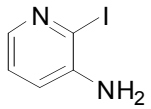
This compound was prepared following a general procedure reported by Pullagurla *et al*. (Pullagurla, 2005) A solution of 4-trimethylsilylbut-3-yn-1-ol (1.0 g, 7.03 mmol) and dry CH_2Cl_2 was cooled to 0°C and Et_3N (1.08 ml, 7.73 mmol) was added dropwise. Methane sulfonyl chloride (600 μl, 7.73 mmol) in CH_2Cl_2 (5 ml) was added to the reaction mixture. The mixture was allowed to stir at room temperature for 1 h, and the reaction mixture was then extracted with brine, CH_2Cl_2 and dried under vacuum. The solid was dissolved in THF (5 ml) and liquid NH_3 was added at -78°C to the reaction mixture and stirred for 3 days at room temperature in a sealed tube. Free amine product was isolated by column chromatography with 5% MeOH in CH_2Cl_2 and starting material, 4-trimethylsilylbut-3-yn-1-ol, could be recovered for further reaction. A solution of the free amine product, 4-trimethylsilylbut-3-yn-1-amine (272 mg, 1.91 mmol), Et_3N (293 μl, 2.10 mmol) in dry CH_2Cl_2 (2 ml) and THF (2 ml) under argon atmosphere was cooled to 0°C and acetic anhydride (199 μl, 2.10 mmol) was added dropwise to the reaction mixture. The reaction mixture was allowed to stir at room temperature for 2 h and was then extracted with brine, CH_2Cl_2 and evaporated to dryness to give a brown solid (41% yield for 2 steps). The product **18** was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) 5.78 (bs, 1H), 3.39 (q, *J*=6.0 Hz, 2H), 2.44 (t, *J*=6.5 Hz), 2.01 (s, 3H), 2.01 (s, 3H), 0.17 (s, 9H)

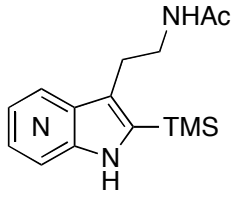
3-amino-2-iodopyridine **19**



This procedure is based on previously reported methods (Klapars and Buchwald, 2002; Oda et al., 2007).

A solution of 2-bromo-3-aminopyridine (0.2 g, 1.16 mmol), NaI (348 mg, 2.32 mmol), CuI (11 mg, 0.06 mmol), and trans-*N*,*N*'-dimethylcyclohexane (19 μl, 0.12 mmol) in 1,4-dioxane (2.5 ml) was heated and stirred at 110°C for 24 h. The reaction mixture was added into water and extracted with ether. The combined organic layers were washed with brine, dried, and concentrated under vacuum. The product **19** was isolated by flash column chromatography on silica gel with 50% EtOAc in hexanes (94% yield as brown crystals). ^{1}H NMR (300 MHz, $CDCl_3$): 7.76 (dd, *J*=1.8Hz, *J*=4.5Hz, 1H), 7.01 (dd, *J*=4.5Hz, *J*=7.8Hz), 6.90 (dd, *J*=1.8Hz, *J*= 7.8Hz), 4.08 (bs, 2H). ^{13}C NMR (75MHz, $CDCl_3$) 144.5, 140.6, 123.9, 120.4, 109.5. LC/Mass (ESI-TOF): m/z calc (M+H): 220.9, found: 220.9.

Typical procedure for 3-(*N*-acetylaminoethyl)-2-trimethylsilyl-1*H*-pyrrole pyridine **20**-**23**



The title compounds were prepared following a general procedure reported by Ujjainwalla et al. (see Figure 2 of manuscript) (Ujjainwalla and Warner, 1998). In a representative procedure, a mixture of 4-amino-3-iodopyridine (121 mg, 0.55 mmol), *N*-

(4-trimethylsiliylbut-3-ynyl)acetamide (300 mg, 1.64 mmol), $Pd(dppf)Cl_2.CH_2Cl_2$ (23 mg, 28 μmol), LiCl (23 mg, 0.55 mmol) and Na_2CO_3 (117 mg, 1.10 mmol) in DMF (1 ml) was heated at 100°C under argon atmosphere and stirred overnight. After cooling, the solution mixture was filtered through celite and DMF was evaporated under high vacuum. The residue was washed by acid-base extraction with 0.01 M HCl aqueous solution (10 ml x 2) and CH_2Cl_2 (10 ml). The water layer was collected and neutralized to pH 8~9 with 1 M NaOH aqueous solution. CH_2Cl_2 (15 ml x 4) was added to the solution and all organic layer were collected and dried under vacuum. The product was isolated by flash column chromatography with 5% MeOH in CH_2Cl_2 to give grey crystals.

3-(*N*-acetylaminoethyl)-2-trimethylsilyl-1*H*-pyrrole[3,2-*b*]pyridine **20** (precursor to **1**)
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.65 (bs, 1H), 8.46 (bs, 1H), 8.33 (d, *J*=4.0 Hz, 1H), 7.63 (d, *J*=8.0 Hz, 1H), 6.99 (dd, *J*=4.5 Hz, *J*=8.0 Hz, 1H), 3.48 (dd, *J*=10.5 Hz, *J*=5.0 Hz, 2H), 3.08 (d, *J*=6.0 Hz, 2H), 1.84 (s, 3H), 0.319 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 146.4, 142.1, 139.3, 131.4, 122.3, 119.0, 116.9, 42.5, 24.5, 23.3, -0.6. Exact mass (ESI-TOF): m/z calc (M+H): 276.1527, found: 276.1533.

3-(*N*-acetylaminoethyl)-2-trimethylsilyl-1*H*-pyrrole[3,2-*c*]pyridine **21** (precursor to **2**)
^{1}H NMR (500 MHz, CD_3OD) δ 8.89 (d, *J*=1.0 Hz, 1H), 8.11 (d, *J*=3.0 Hz, 1H), 7.45 (dd, *J*=6.0 Hz, *J*=1.0 Hz, 1H), 3.42 (t, *J*=7.5 Hz, 2H), 3.09 (t, *J*=7.5 Hz, 2H), 1.84 (s, 3H), 0.319 (s, 9H). ^{13}C NMR (125MHz, CD_3OD) δ 173.4, 146.0, 145.0, 136.2, 132.1, 127.0, 125.9, 110.0, 42.1, 26.9, 22.9, -0.6. Exact mass (ESI-TOF): m/z calc (M+H): 276.1527, found: 276.1532.

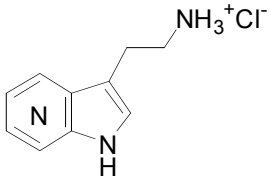
3-(*N*-acetylaminoethyl)-2-trimethylsilyl-1*H*-pyrrole[2,3-*c*]pyridine **22** (precursor to **3**)

^{1}H NMR (500 MHz, CD_3OD) δ 9.02 (s, 1H), 8.19 (d, *J*=6.5 Hz, 1H), 8.13 (dd, *J*=6.0 Hz, *J*=0.5 Hz, 1H), 3.42 (t, *J*=8.0 Hz, 2H), 3.08 (d, *J*=8.0 Hz, 2H), 1.93 (s, 3H), 0.50 (s, 9H). ^{13}C NMR (125MHz, CD_3OD) δ 173.2, 152.7, 138.3, 135.2, 129.5, 128.5, 123.7, 116.6, 41.7, 26.6, 23.0, -0.65. Exact mass (ESI-TOF): m/z calc (M+H): 276.1527, found: 276.1536.

3-(*N*-acetylaminoethyl)-2-trimethylsilyl-1*H*-pyrrole[2,3-*b*]pyridine **23** (precursor to **4**)

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.65 (bs, 1H), 8.30 (dd, *J*=7.5 Hz, *J*=1.5 Hz, 1H), 7.95 (d, *J*=7.5 Hz, 1H), 7.05 (dd, *J*=8.0 Hz, *J*=4.5 Hz, 1H), 5.65 (bs, 1H), 3.53 (q, *J*=6.5 Hz, 2H), 3.04 (t, *J*=7.0 Hz, 2H), 1.92 (s, 3H), 0.42 (s, 9H). ^{13}C NMR (125MHz, $CDCl_3$) δ 170.2, 150.8, 143.8, 135.5, 127.4, 121.2, 120.2, 115.6, 40.9, 26.5, 23.6, -0.26. Exact mass (ESI-TOF): m/z calc (M+H): 276.1527, found: 276.1538.

Typical procedure for 4-, 5-, 6- and 7-azaindole tryptamine (HCl) **1**-**4**



To remove the silyl group and generate the hydrochloride salt, a solution of 3-(*N*-acetylaminoethyl)-2-trimethylsilyl-1*H*-pyrrole[3,2-*c*]pyridine (90 mg, 0.327 mmol) and conc. HCl aq. (~32%, 5 ml) was heated at reflux condition for 48 h. After cooling, the reaction mixture was evaporated under vacuum, saturated in ethanol and crystallized by

slow solvent evaporation to give brown crystals (49% ~ 56% yield for 2 steps). The hydrochloride salts were used without further purification for all subsequent steps.

3. Supplemental References

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