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An Alternate Synthesis of the Tat-Antagonist 7-Chloro-*N*-methyl-5-(1*H*-pyrrol-2-yl)-3*H*-1,4-benzodiazepin-2-amine

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Abstract—An alternative synthesis of 7-chloro-*N*-methyl-5-(1*H*-pyrrol-2-yl)-3*H*-1,4-benzodiazepin-2-amine, the compound that inhibits gene expression by HIV-1 at the level of transcriptional transactivation by Tat, has been developed. The process is based on ring expansion of 6-chloro-2-chloromethyl-4-(1*H*-pyrrol-2-yl)quinazoline 3-oxide which leads to the corresponding benzodiazepine Ro24-7429. Quinazoline 3-oxide formation in the presence of boron trifluoride gives a tetracyclic system containing a 2,2-difluoro-1,3,6,2-oxadiazaborine ring that survives ring expansion to 13-chloro-5,5-difluoro-9-(methylamino)-5*H*-pyrrolo[1',2':3,4]-1,3,6,2-oxadiazabora[6,5-*d*]-8*H*-1,4-benzodiazepin-7-ium hydroxide inner salt. This unusual benzodiazepine does not significantly inhibit Tat-mediated gene expression by HIV-1.

A search for agents that inhibit replication of the human immunodeficiency virus (HIV-1) by inhibition of the transcription transactivator Tat led to 7-chloro-5-(1*H*-pyrrol-2-yl)-3*H*-1,4-benzodiazepin-2-one (**10**, Ro5-3335).[1] The amidine derivative **7** (Ro24-7429) exhibited an improved toxicological profile[2,3] and was chosen for further evaluation. It exhibited broad activity against several strains of HIV-1 in different cell lines, peripheral blood lymphocytes and macrophages and inhibited viral replication in acute and chronic infections *in vitro*.[4]

The currently used synthesis of **7** is based on the amidination of **10**, a step that has not yet been achieved in high yield. We have now synthesized **7** by ring expansion of **5** with methylamine which eliminates the intermediacy of **10**. The quinazoline oxide **5** was prepared by oxidation of **3** and by an *in situ* cyclization of **4**. The initial formation of an *E/Z*-oxime mixture (**2**), estimated to be in a ratio of 1:2 on the basis of the ^{1}H NMR spectrum, is not expected to influence the quinazoline yield in view of precedented isomerization during the cyclization reaction.[5] Thus, an *E/Z*-mixture of **4**, obtained in a ratio of 2:3 in a separate experiment, was subjected to cyclization conditions. Upon work-up, only *Z*-isomer could be isolated as unreacted material. In the absence of isomerization during the reaction, the unreactive *E*-isomer would be expected to accumulate. The final step of this route consists of the hydrogenolysis of the resulting benzodiazepine 4-oxide as illustrated in Scheme 1.

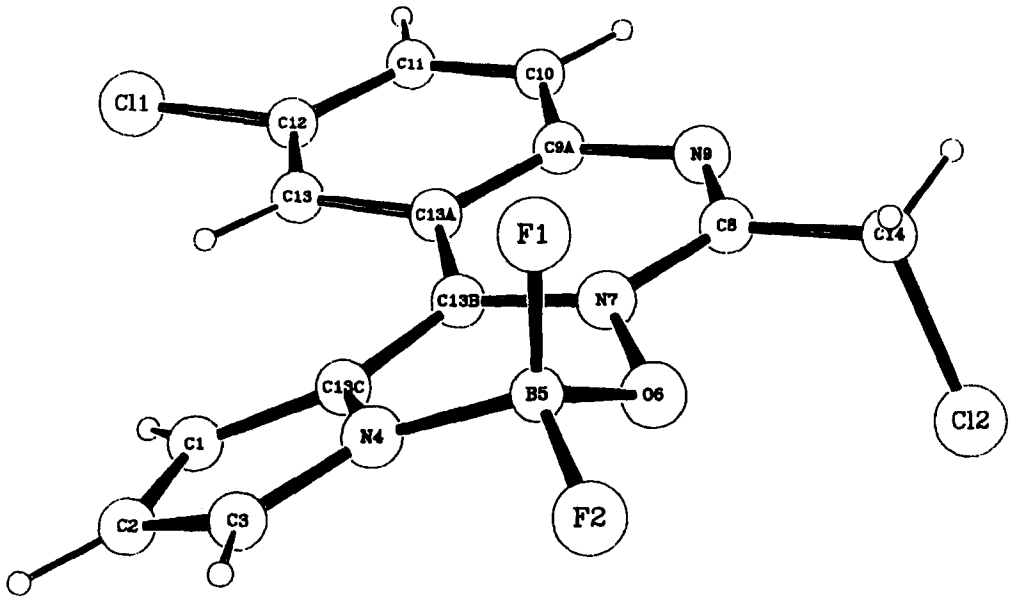
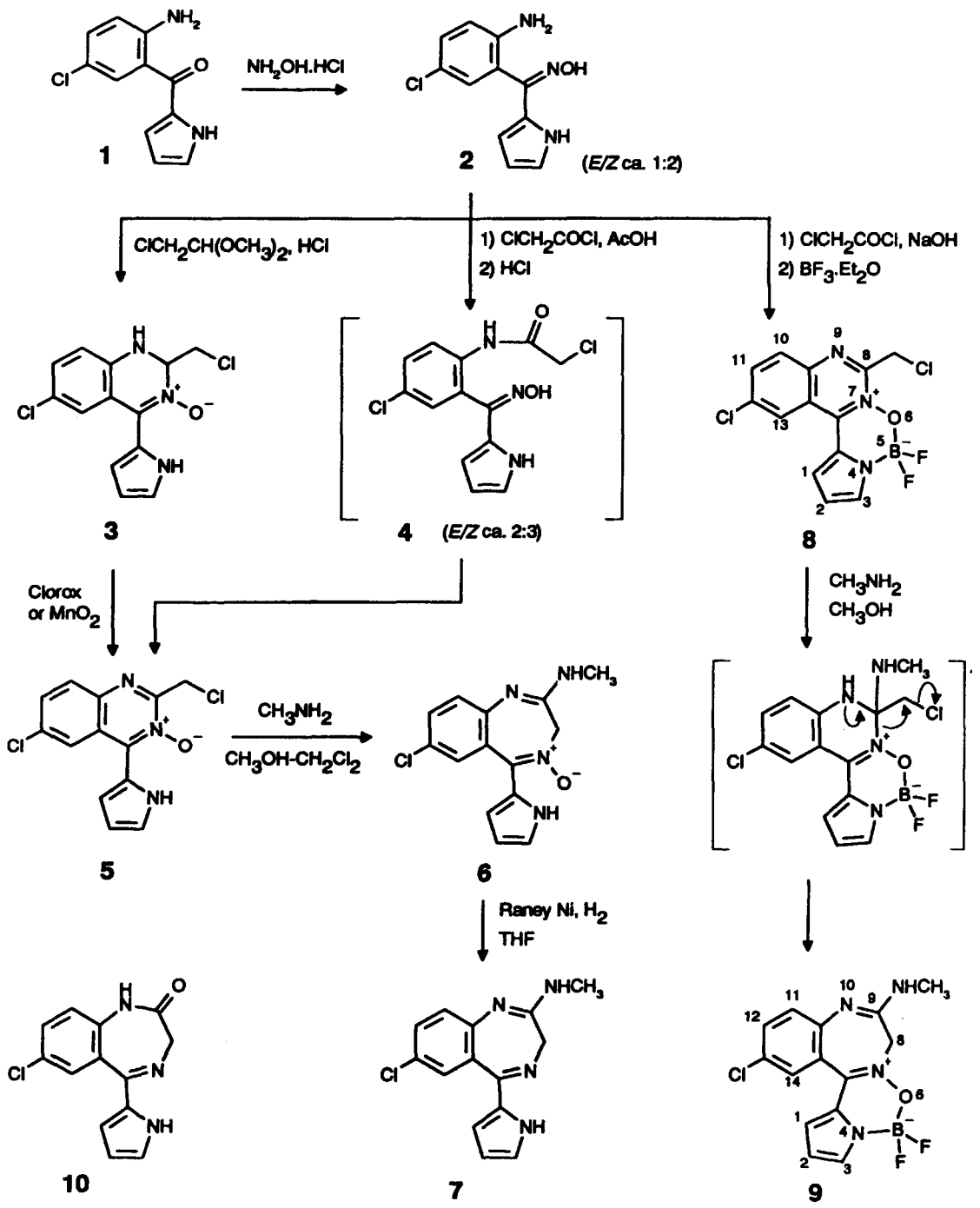


Figure 1. Stereostructure of compound **8**.



Scheme 1.

An attempt to catalyze the generation of quinazoline 3-oxide from **4** by boron trifluoride etherate proceeded with the concomitant loss of hydrogen fluoride and formation of **8** containing a 2,2-difluoro-1,3,6,2-oxadiazaborine ring. The stereostructure of **8**, obtained by single-crystal Roentgen diffraction analysis is shown in Figure 1.

Ring expansion of **8** with methylamine to the seven-membered amidine **9**, initiated by the addition of the amine to the imino carbon is facilitated by the presence of the iminium–carbon bond. The actual ring enlargement could subsequently proceed either in a two-step process proposed for the *N*-oxide series,[5] or by a nucleophilic 1,2-shift involving the iminium–carbon bond as the migrating entity, as indicated in Scheme 1. In view of the modest yield of this reaction, conversion of **9** to **7** was not attempted. Compound **9** was virtually inactive in the aforementioned *in vitro* tests.

Experimental

(E,Z)-(2-Amino-5-chlorophenyl)-1H-pyrrol-2-yl-

methanone oxime (2). A mixture of (2-amino-5-chlorophenyl)-1*H*-pyrrol-2-yl-methanone[6] (8.8 g, 0.04 mol), hydroxylamine hydrochloride (11.12 g, 0.16 mol) and pyridine (150 mL) was stirred and heated at reflux temperature under argon for 18 h. The solution was evaporated and the residue partitioned between EtOAc and saturated NaCl solution. The EtOAc phase was washed with brine, dried ($MgSO_4$) and evaporated to yield a 1:2 mixture of *E*- and *Z*-isomers, together with 14% of pyridine (0.5 mol), as a dark paste which was used without further purification; 400 MHz ^{1}H NMR ($CDCl_3$): δ 3.97 (2H, *br s*, NH_2), 6.12, 6.19, 6.84 (3 × 1/3H, *br s*, *m*, *br s*, H-4', H-3', H-5' of *E*-**2**), 6.24, 6.26, 7.02 (3 × 2/3H 3 *br s*, H-4' H-3', H-5' of *Z*-**2**), 6.69 (2/3H, *d*, H-3 of *Z*-**2**, J_o = 8.5 Hz), 6.72 (1/3H, *d*, H-3 of *E*-**2**, J_o = 8.5 Hz), 7.15 (2/3H, *dd*, H-4 of *Z*-**2**, J_m = 2 and J_o = 8.5 Hz), 7.19 (1/3H, *d*, H-6 of *E*-**2**, J_m = 2 Hz), 7.19 (1/3H, *dd*, H-4 of *E*-**2**, J_m = 2 and J_o = 8.5 Hz), 7.24 (2/3H, *d*, H-6 of *Z*-**2**, J_m = 2 Hz), 9.20 (1/3H, *br s*, NH of *E*-**2**,), 10.46 (2/3H, *br s*, NH of *Z*-**2**).

(±)-6-Chloro-2-chloromethyl-1,2-dihydro-4-(1H-pyrrol-2-yl)-quinazoline 3-oxide (3). A mixture of chloroacetaldehyde dimethylacetal (5.33 g, 0.043 mol) and HCl (1.4 N, 8.5 mL) was heated at reflux temperature for 15 min, cooled and added to the oxime mixture **2** (5.03 g, 0.0183 mol), previously dissolved in EtOH (40 mL). After 1.5 h the mixture was neutralized with 5% aq. Na_2CO_3 solution and partitioned between CH_2Cl_2 and H_2O. The aq. phase was washed once with CH_2Cl_2 and the combined CH_2Cl_2 layers were evaporated. The resulting foam was flash chromatographed (4:1 CH_2Cl_2:EtOAc, 5 cm column diam) to furnish **3** as a crystalline substance (3.8 g, 70%) which was used directly in the next step. For analysis, a sample was recrystallized from CH_2Cl_2:EtOAc – pentane to give yellow prisms, mp 163–164 °C, 400 MHz ^{1}H NMR ($CDCl_3$): δ 3.71 (1H, *dd*, J_{vic} = 10 and J_{gem} = 11 Hz, CH of CH_2Cl), 4.06 (1H, *dd*, J_{vic} = 3 and J_{gem} = 11 Hz, CH of CH_2Cl), 4.98 (1H, *br s*, NH), 5.14 (1H, *m*, H-2), 6.42 (1H, *m*, H-4'), 6.87 (1H, *m*, H-3'), 6.88 (1H, *d*, H-8, J_o = 8.5 Hz), 7.07 (1H, *m*, H-5'), 7.26 (1H, *dd*, H-7, J_o = 8.5, J_m = 2 Hz), 7.82 (1H, *d*, H5, J_m = 2Hz), 12.53 (1H, *br s*, NH'); mass spectrum, m/z (rel intensity): 293 (92, $[M]^+$), 277 (87, [M–O]), 276 (100, [M–OH]), 240 (62, [M–OH–HCl]), 228 (28, [M–O–CH_2Cl]), 192 (36, [M–O–CH_2Cl–HCl]). Anal. calcd for $C_{13}H_{11}Cl_2N_3O$: C, 52.39; H, 3.70; N, 13.86. Found: C, 52.72; H, 3.74; N, 14.19.

6-Chloro-2-chloromethyl-4-(1H-pyrrol-2-yl)-quinazoline 3-oxide (5). From **2**: chloroacetyl chloride (3.5 mL, 0.044 mol) was added to a stirred solution of the oxime mixture **2** (9.4 g, 0.03 4 mol) in HOAc (125 mL). The mixture was stirred at room temperature for 2.75 h and was then saturated with gaseous HCl. After warming to 55 °C, the flask was stoppered and kept at room temperature for 60 min. Evaporation of the mixture furnished a residue that was partitioned between CH_2Cl_2 and aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and evaporated. Flash chromatography (2.5" diam column, 10:1 CH_2Cl_2:EtOAc) of the brown residue gave **5** as yellow needles (3.3 g, 33%), mp 161–166 °C, 400 MHz ^{1}H NMR ($CDCl_3$): δ 5.08 (2H, *s*, CH_2Cl), 6.60 (1H, *m*, H-4'), 7.23 (1H, *m*, H-5'), 7.71 (1H, *dd*, H-7, J_o = 9 and J_m = 2 Hz), 7.95 (1H, *d*, H-8, J_o = 9 Hz), 8.51 (1H, *d*, H-5, J_m = 2 Hz), 12.87 (1H, *br s*, NH). Anal. calcd for $C_{13}H_9Cl_2N_3O$: C, 53.08; H, 3.08; N, 14.29. Found: C, 52.68; H, 3.18; N, 14.26. Continued elution (15:85, CH_2Cl_2:EtOAc) gave unreacted *Z*-**4** (1.45 g), cream colored prisms from CH_2Cl_2 pentane, mp 161–163 °C; 400 MHz ^{1}H NMR (DMSO-d_6): δ 4.22 (2H, *s*, CH_2Cl), 6.00 (1H, *m*, H-4'), 6.09 (1H, *m*, H-3'), 6.97 (1H, *m*, H-5'), 7.37 (1H, *d*, H-6, J_m = 2.5 Hz), 7.52 (1H, *dd*, H-4, J_o = 9 and J_m = 2.5 Hz), 7.99 (1H, *d*, H-3, J_o = 9 Hz), 9.76 (1H, *s*, NHCO), 11.41 (1H, *br s*, NH), 11.88 (1H, *s*, NOH); (+)FAB mass spectrum m/z (rel intensity) 312 (22, [M+1]).

From **3**: by oxidation with NaOCl: A mixture of **3** (296 mg, 1 mmol), CH_2Cl_2 (5 mL), Clorox (3 mL, *ca* 5% NaOCl) was stirred as a two-phase system for 2.5 h; another 3-mL quantity of Clorox was added and stirring was continued for 2 h. The aqueous phase was extracted with CH_2Cl_2 and the combined organic layers were washed with H_2O, dried (Na_2SO_4) and evaporated. Upon trituration with MeOH–Et_2O, the resulting brown solids crystallized (210 mg, 70%). Recrystallization from EtOAc – pentane gave yellow needles, mp 163–165 °C.

By oxidation with manganese(IV) oxide: a suspension of activated manganese(IV) oxide (2.5 g) in CH_2Cl_2 (75 mL) containing **3** (0.5 g, 1.69 mmol) was stirred at reflux temperature for 3.5 h. Evaporation of the filtrate gave mustard-colored solids (0.47 g) which were purified as described above to furnish **4** in comparable yield.

(E,Z)-2-Chloro-N-[4-chloro-2-[(hydroxyimino)-1H-pyrrol-2-ylmethyl]phenyl]acetamide (4). To a vigorously stirred mixture of oxime **2** (8.0 g, 34 mmol), CH_2Cl_2 (160 mL), and H_2O (52 mL) was added simultaneously, at –3 to 0 °C, over a 30 min period, chloroacetyl chloride (3.82 g, 34 mmol) in CH_2Cl_2 (20 mL) and 2 N NaOH (17 mL). The mixture was stirred at room temperature for 1 h, the aq. phase was washed once with CH_2Cl_2 and the combined organic layers were dried (Na_2SO_4) and evaporated to give crude **4** (8.8 g). Flash chromatography (1:2 Me_2CO hexane) gave a 2:3 *E/Z* mixture; 400 MHz ^{1}H NMR (DMSO-d_6): δ 4.22 (0.4H, *s*, CH_2Cl of *E*-**4**), 4.32 (0.6H, *s*, CH_2Cl of *Z*-**4**), 5.76, 6.04, 6.86 (3 × 0.4H, *br s*, *m*, *br s*, H-3',H-4', H-5' of *E*-**4**), 6.00, 6.09, 6.86 (3 × 0.6H, *br s*, *m*, *br s*, H-3',H-4', H-5' of *Z*-**4**), 7.27 (0.4H, *d*, H-6 of *E*-**4**, J_m = 2.5 Hz), 7.37 (0.6H, *d*, H-6 of *Z*-**4**, J_m = 2.5 Hz), 7.50–7.56 (1H, *m*, H-4 of *E*-**4** and *Z*-**4**) 7.89 (0.4H, *d*, H-3 of *E*-**4**, J_o = 8.5 Hz), 7.99 (0.6H, *d*, H-3 of *Z*-**4**, J_o = 8.5 Hz), 9.14, 11.22, 11.47 (3 × 0.4H, *s*, *br s*, *s*, NHCO, NH and NOH of *E*-**4**), 9.75, 11.40, 11.88 (3 × 0.6H *s*, *br s*, *s*, NHCO, NH and NOH of *Z*-**4**).

7-Chloro-N-methyl-5-(1H-pyrrol-2-yl)-2H-1,4-benzodiazepin-2-amine 4-oxide (6). A solution of **5** (0.50 g, 17 mmol) in CH_2Cl_2 (6 mL) and methylamine in MeOH (30% w/v, 6 mL) was stirred in a stoppered flask for 2 h at room temperature and evaporated. The residue was treated with tetrahydrofuran, the resulting mixture was filtered and the filtrate was concentrated and diluted with a small quantity of hexane to afford **6** as tan prisms (50.9%). Recrystallizations from CH_2Cl_2–pentane and tetrahydrofuran–pentane gave pure **6**, mp 258–260 °C; 400 MHz ^{1}H NMR (DMSO-d_6): δ 2.79 (3H, *d*, $J_{NH,Me}$ = 4.5 Hz), 4.33 and 4.36 (2H, AB, H-3, $J_{3a,3b}$ = 13 Hz), 6.22, 6.25 (1H each, *m*, H-3', H-4'), 7.01 (1H, *m*, H-5'), 7.14 (1H, *d*, H-9, J_o = 8.5 Hz), 7.40 (1H, *dd*, H-8, J_o = 8.5, J_m = 2.5 Hz), 7.57 (1H, *d*, H-6, J_m = 2.5 Hz), 12.34 (1H, *br s*, NH); mass spectrum, m/z (rel intensity): 288 (80, $[M]^+$), 272 (81, [M–O]), 271 (100, [M–OH]), 242 (44, [M–O–$NHCH_3$]), 236 (50, [M–OH–Cl]). Anal. calcd for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; N, 19.40. Found: C, 58.25; H, 4.51; N, 19.18.

7-Chloro-N-methyl-5-(1H-pyrrol-2-yl)-3H-1,4-benzodiazepin-2-amine (7). A mixture of **6** (373 mg, 13 mmol), tetrahydrofuran (15 mL) and Raney nickel (1.2 g, slurried in water) was stirred under 1 atm of hydrogen pressure. Hydrogen uptake ceased after 15 min and TLC analysis (4:1, EtOAc:EtOH) indicated completion of the reaction. The suspension was filtered, and the filtrate was evaporated to furnish a crystalline residue (89.6%) which was recrystallized twice from tetrahydrofuran–pentane to give pure **7**, mp 255–257 °C; 400 MHz ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 2.86 (*br s*, CH_3N+HDO), 3.45 (1H, *d*, H-3a, $J_{3a,3b}$ = 10.5 Hz), 4.44 (1H, *d*, H-3b, $J_{3a,3b}$ = 10.5 Hz), 6.19 (1H, *m*, H-4'), 6.32 (1H, *m*, H-3'), 6.54 (1H, *d*, $J_{NH,Me}$ = 4 Hz), 6.91 (1H, *br s*, H-5'), 7.13 (1H, *d*, H-9, J_o = 9 Hz), 7.33 (1H, *dd*, H-8, J_o = 9 and J_m = 2.5 Hz), 7.70 (1H, *d*, H-8, J_o = 9 Hz, 10.10 (1H, *br s*, NH); mass spectrum, m/z (rel intensity): 272 (100, $[M]^+$), 271 (62, [M–H]), 257 (19, [M–CH_3]), 244 (33, [M–H–HCN]), 242 (29, [M–$NHCH_3$]), 236 (25, [M–HCl]), 208 (18, [M–HCl–H–HCN]). Anal. calcd for $C_{14}H_{13}ClN_4$: C, 61.65; H, 4.80; N, 20.54. Found: C, 61.33; H, 4.80, N, 20.34.

12-Chloro-8-chloromethyl-5,5-difluoro-5H-pyrrolo-[1',2':3,4]-1,3,6,2-oxadiazabora[6,5c] quinazolin-7-ium hydroxide inner salt (8). A mixture of **2** (10 g, 0.0424 mol, mixture of isomers), Et_2O (100 mL), CH_2Cl_2 (10 mL) and H_2O (25 mL) was stirred vigorously at 0 °C. Chloroacetyl chloride (3.5 mL, 0.044 mol) and 2 N NaOH (22 mL) was added simultaneously over a 10 min period. The thick suspension was stirred for 30 min and chloroacetyl chloride (1 mL) and 2 N sodium hydroxide (6.5 mL) were added. The mixture was refrigerated overnight and filtered to remove some diacylated material. The organic phase of the filtrate was separated, washed with brine, dried (Na_2SO_4) andevaporated to yield crude **4** as a tan solid (6 g, 45%). A solution of 5.9 g of this material in toluene (200 mL), 1,1,1-trichloroethane (50 mL) and boron trifluoride etherate (5 mL) was stirred under Ar at 80 °C for 5 h. The yellow solution, decanted from some tarry substance was washed with saturated sodium hydrogen carbonate solution and water, dried (magnesium sulfate) and evaporated to give crude **8** as a yellow solid (3.4 g, 54%). Recrystallizations from EtOH–CH_2Cl_2, CH_2Cl_3–pentane and tetrahydrofuran–pentane gave yellow prisms, mp 221–223 °C; 400 MHz ^{1}H NMR ($CDCl_3$): δ 5.06 (2H, *s*, CH_2Cl), 6.79 (1H, *dd*, H-2, $J_{1,2}$ = 4 and $J_{2,3}$ = 2 Hz), 7.64 (1H, *d*, H-1, $J_{1,2}$ = 4 Hz), 7.69 (1H, *d*, H-3, $J_{2,3}$ = 2 Hz), 7.95 (1H, *dd*, H-11, J_o = 9 and J_m = 2.5 Hz), 8.02 (1H, *d*, H-10, J_o = 9 Hz), 8.59 (1H, *d*, H-13, J_m = 2.5 Hz); mass spectrum, m/z (rel intensity): 341 (100, $[M]^+$), 324 (12, [M–OH]), 307 (13, [M–Cl]),292 (8, [M–CH_2Cl]), 276 (27, [M–CH_2Cl–O]). A crystal from tetrahydrofuran (0.10 × 0.32 × 0.40 mm) was analyzed (Cu K_α radiation, ω-2θ scans; 2861 independent reflections for θ < 75, 2560 used [$I > 3.0\sigma(I)$], R = 0.046 and wR = 0.065), found to be triclinic, space group $P1$, a = 8.945(1), b = 9.319(5), c = 9.401(2) Å, α = 107.88(3), β = 102.45(1), γ = 102.51(2)°, Z = 2, d_{calc} = 1.637 g cm^{-3}, μ (CuKa) = 45.5 cm^{-1}. The resulting structure is shown in Figure 1.

13-Chloro-5,5-difluoro-9-(methylamino)-5H-pyrrolo-[1',2':3,4]-1,3,6,2-oxadiazabora[6,5-d]-8H-1,4-benzodiazepin-7-ium hydroxide inner salt (9). A solution of **8** (0.83 g, 24 mmol) containing 30 % (w/v) of methylamine in methanol (7 mL) was kept in a stoppered flask for 5 h and evaporated. The residue was flash-chromatographed (3:1 CH_2Cl_2:EtOH, 15 mm column diam) to furnish **9** as yellow crystals (0.58 g, 71%), recrystallized from CH_2Cl_2 – hexane and Et_2O–pentane gave mp 214–216 °C; 400 MHz ^{1}H NMR ($CDCl_3$): δ 3.00, 3,01 (3H, $NHCH_3$, $J_{NH,Me}$ = 5 Hz), 4.25 (1H, *d*, H-8a, $J_{8a,8b}$ = 14.5 Hz), 4.55 (1H, *d*, H-8b, $J_{8a,8b}$ = 14.5 Hz), 5.69 (1H, *br d*, NH, $J_{NH,Me}$ = 5 Hz), 6.55 (1H, *dd*, H-2, $J_{1,2}$ = 4 and $J_{2,3}$ = 2 Hz), 6.83 (1H, *d*, H-1, $J_{1,2}$ = 4 Hz), 7.28 (1H, *d*, H-11, J_o = 9 Hz), 7.48 (1H, *d*, J_{23} = 2 Hz), 7.53 (1H, *dd*, H-12, J_o = 9 and J_m = 2.5 Hz), 7.82 (1H, *d*, H-14, J_o = 9 Hz). Anal. calcd for $C_{14}H_{12}B,Cl,F,N_4O$: C, 49.97; H, 3.59; N, 16.65. Found: C, 49.93; H, 3.62; N, 16.30.

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