Supporting Information

In situ Selection of Lead Compounds by Click Chemistry: Target-guided Optimization of Acetylcholinesterase Inhibitors

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CAUTION! All of the compounds described here (and especially the most potent polyvalent inhibitors) are potentially neurotoxic. They must be handled with extreme care by trained personnel. Azide-containing compounds, particularly those lower in saturated carbon and oxygen content, are potentially explosive and must be handled with care.

General. Reactions requiring anhydrous conditions were run under nitrogen in glassware flame dried under vacuum. Reagents were purchased from Acros, Aldrich, and Lancaster and were used as received. Reaction progress was monitored by TLC using Merck silica gel 60 F-254 plates with detection by UV or by immersion in staining solution ($KMnO_4$). Silica gel 60 (Merck 40–63 μm) was used for column chromatography.

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian Inova-400 spectrometer. Proton magnetic resonance (^{1}H NMR) spectra were recorded at 400 MHz. Data are presented as follows: chemical shift (ppm), multiplicity (*s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *quin* = quintet, *m* = multiplet), coupling constant (Hz) and integration. Carbon magnetic resonance (^{13}C NMR) spectra were recorded at 100 MHz. Data for ^{13}C NMR are reported in terms of chemical shifts (ppm). GC was performed on a Hewlett Packard 5890 Series II with an FID detector using a J&W Scientific DB-5 column (0.32 mm x 30 m). HPLC was performed on an Agilent 1100 LC/MSD with an Agilent 1100 SL mass spectrometer, using four different elution solvents: Solvent A (0.05 % TFA in H_2O), solvent B (0.05 % TFA in CH_3CN), solvent C (1 % Me_3N/formic acid in H_2O, pH = 7.5) and solvent D (CH_3CN).

Regioisomer determination. The sensitive LC/MS-SIM method was employed for determining the regioisomer distribution of the in situ click chemistry products **(*R*)-TZ2PIQ-A5**, **(*S*)-TZ2PIQ-A5**, **(*R*)-TZ2PIQ-A6** and **(*S*)-TZ2PIQ-A6**. The assignment was accomplished by comparing the retention times of the in situ products with authentic samples, prepared by the addition of magnesium acetylides to azides,[1] yielding selectively the syn-isomers and copper-catalyzed azide/acetylene reaction,[2,3] yielding pure anti-regioisomers. The tacrine azide **TZ2** was dissolved in MeOH and added to 2-8 μM solutions of eel AChE or mouse AChE in buffer (2 mM ammonium citrate, 100 mM NaCl, pH=7.3-7.5) followed immediately by one of the acetylene components and mixed. The final concentrations were as follows:

eel or mouse AChE: 2-8 µM; tacrine azide (**TZ2**): 19 µM, acetylene component: 196-294 µM. Each reaction mixture was incubated at 37 °C for at least six days. The in situ reaction mixtures were injected directly (2-50 µL) into the LC/MS system as well as co-injected with the reference compounds. The chromatography was performed on a Zorbax SIL column (4.6 mm x 25 cm), preceded by a Phenomenex C18 ODS guard column, at a flow rate of 0.6 mL/min. The MS detector settings used were the same as for the in situ screening. The eluent used was C:D 15:85 for **TZ2PIQ-A5** or C:D 10:90 for **TZ2PIQ-A6**. Comparison of all traces revealed that the in situ click chemistry reactions were highly selective for *syn*-triazole isomers.

Figure S1. Regioisomer determination for mouse AChE-derived in situ hits. The in situ product, **(*S*)-TZ2PIQ-A5**, was compared by LC/MS-SIM to authentic samples, prepared by the Cu(I)-catalyzed and Mg-mediated reactions, which give *anti*- and *syn*-**TZ2PIQ-A5**, respectively. **A)** *anti*-**(*S*)-TZ2PIQ-A5** prepared by the Cu(I)-catalyzed reaction; **B)** co-injection of **(*S*)-TZ2PIQ-A5** prepared by the in situ click reaction and *anti*-**(*S*)-TZ2PIQ-A5** prepared by the Cu(I)-catalyzed reaction; **C)** in situ click reaction; **D)** co-injection of the in situ click reaction and *syn*-**(*S*)-TZ2PIQ-A5** prepared by the Mg-mediated reaction. **E)** *syn*-**(*S*)-TZ2PIQ-A5** prepared by the Mg-mediated reaction.

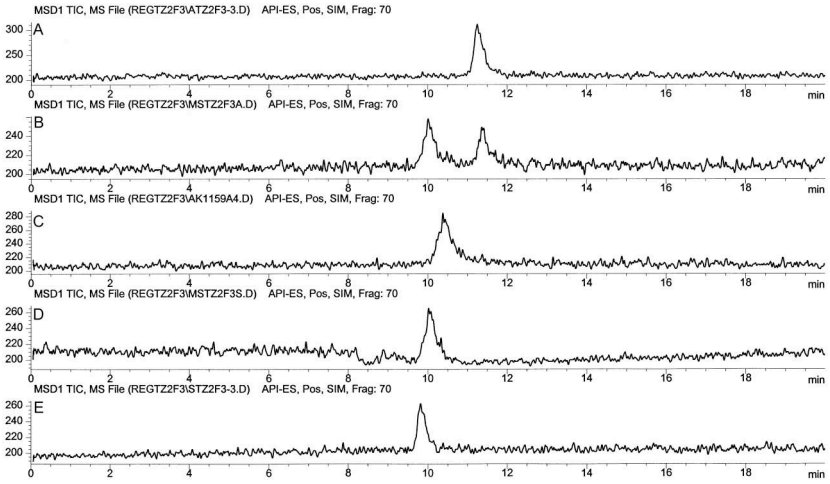


Figure S2. Regioisomer determination for eel AChE-derived in situ hits. The in situ product, **(*R*)-TZ2PIQ-A5**, was compared by LC/MS-SIM to authentic samples, prepared by the Cu(I)-catalyzed and Mg-mediated reactions. **A)** *anti*-**(*R*)-TZ2PIQ-A5** prepared by the Cu(I)-catalyzed reaction; **B)** co-injection of **(*R*)-TZ2PIQ-A5** prepared by the in situ click reaction and *anti*-**(*R*)-TZ2PIQ-A5** prepared by the Cu(I)-catalyzed reaction; **C)** in situ click reaction; **D)** co-injection of the in situ click reaction and *syn*-**(*R*)-TZ2PIQ-A5** prepared by the Mg-mediated reaction. **E)** *syn*-**(*R*)-TZ2PIQ-A5** prepared by the Mg-mediated reaction.

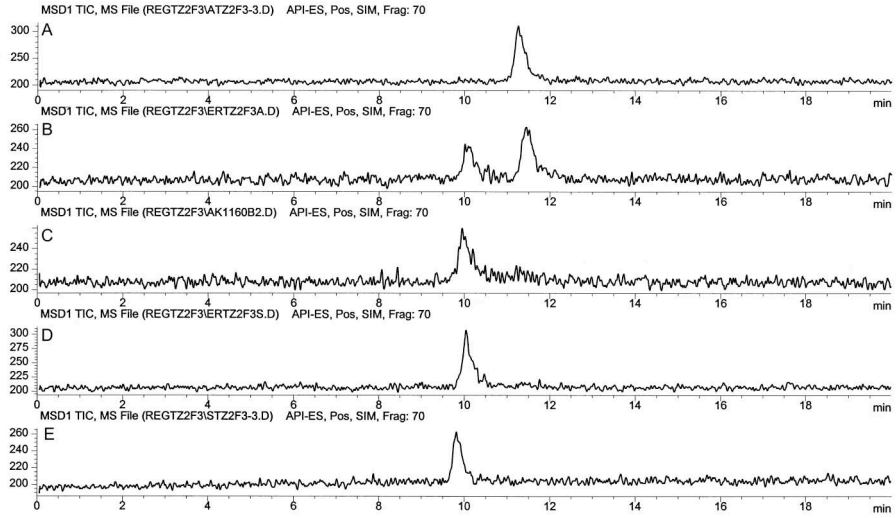


Figure S3. Regioisomer determination for eel AChE-derived in situ hits. The in situ product, **(*S*)-TZ2PIQ-A5**, was compared by LC/MS-SIM to authentic samples from the Cu(I)-catalyzed and Mg-mediated reactions. **A)** *anti*-**(*S*)-TZ2PIQ-A5** prepared by the Cu(I)-catalyzed reaction; **B)** co-injection of **(*S*)-TZ2PIQ-A5** prepared by the in situ click reaction and *anti*-**(*S*)-TZ2PIQ-A5** prepared by the Cu(I)-catalyzed reaction; **C)** in situ click reaction; **D)** co-injection of the in situ click reaction and *syn*-**(*S*)-TZ2PIQ-A5** prepared by the Mg-mediated reaction. **E)** *syn*-**(*S*)-TZ2PIQ-A5** prepared by the Mg-mediated reaction

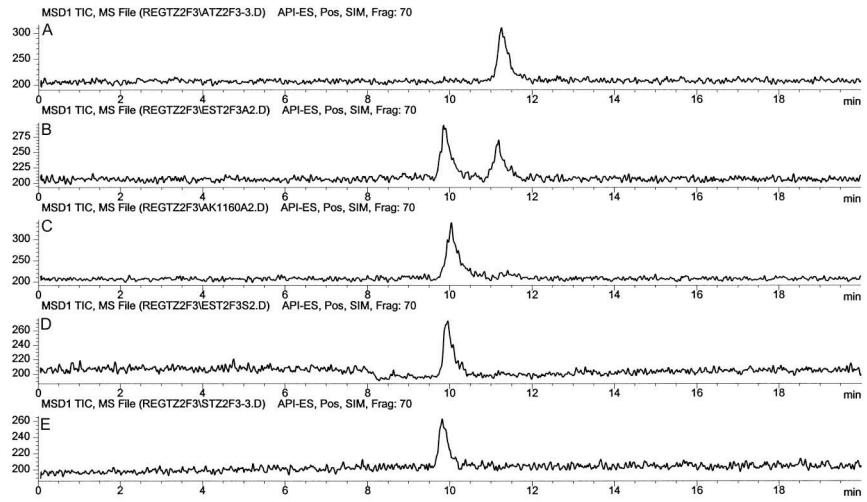


Figure S4. Regioisomer determination for mouse AChE-derived in situ hits. The in situ product, **(*R*)-TZ2PIQ-A6**, was compared by LC/MS-SIM to authentic samples from the Cu(I)-catalyzed and Mg-mediated reactions. **A)** *anti*-**(*R*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **B)** co-injection of **(*R*)-TZ2PIQ-A6** prepared by the in situ click reaction and *anti*-**(*R*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **C)** in situ click reaction; **D)** co-injection of the in situ click reaction and *syn*-**(*R*)-TZ2PIQ-A6** prepared by the Mg-mediated reaction. **E)** *syn*-**(*R*)-TZ2PIQ-A6** prepared by the Mg-mediated reaction.

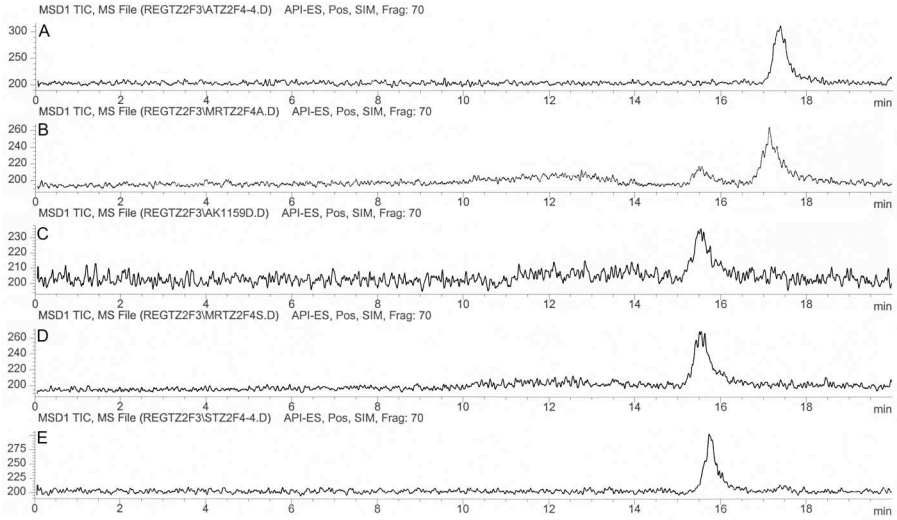


Figure S5. Regioisomer determination for mouse AChE-derived in situ hits. The in situ product, **(*S*)-TZ2PIQ-A6**, was compared by LC/MS-SIM to authentic samples from the Cu(I)-catalyzed and Mg-mediated reactions. **A)** *anti*-**(*S*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **B)** co-injection of **(*S*)-TZ2PIQ-A6** prepared by the in situ click reaction and *anti*-**(*S*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **C)** in situ click reaction; **D)** co-injection of the in situ click reaction and *syn*-**(*S*)-TZ2PIQ-A6** prepared by the Mg-mediated reaction. **E)** *syn*-**(*S*)-TZ2PIQ-A6** prepared by the Mg-mediated reaction.

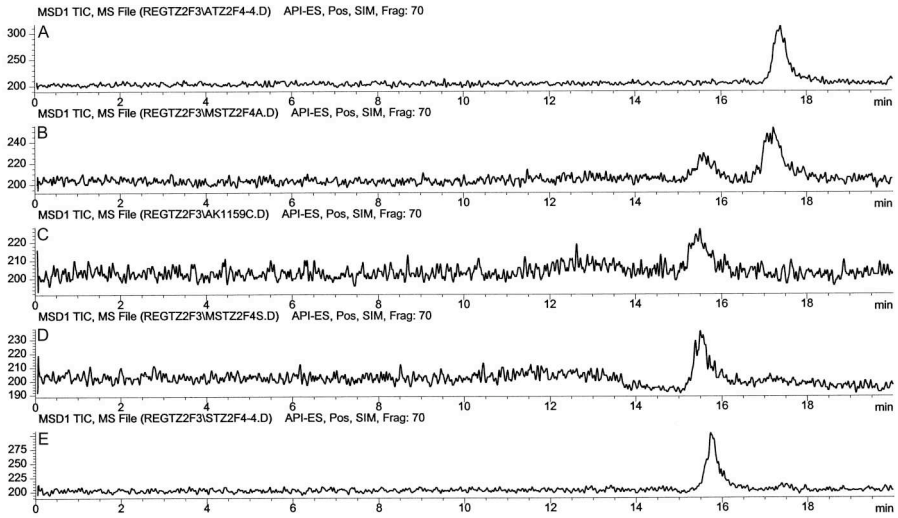


Figure S6. Regioisomer determination for eel AChE-derived in situ hits. The in situ product, **(*R*)-TZ2PIQ-A6**, was compared by LC/MS-SIM to authentic samples from the Cu(I)-catalyzed and Mg-mediated reactions. **A)** *anti*-**(*R*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **B)** co-injection of **(*R*)-TZ2PIQ-A6** prepared by the in situ click reaction and *anti*-**(*R*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **C)** in situ click reaction using ~8 M purified AChE; **D)** *syn*-**(*R*)-TZ2PIQ-A6** prepared by the Mg-mediated reaction.

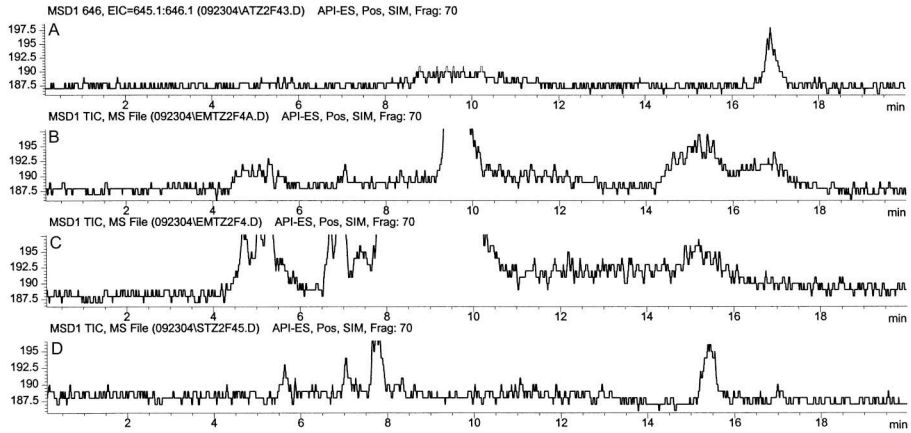


Figure S7. Regioisomer determination for eel AChE-derived in situ hits. The in situ product, **(*S*)-TZ2PIQ-A6**, was compared by LC/MS-SIM to authentic samples from the Cu(I)-catalyzed and Mg-mediated reactions. **A)** *syn*-**(*S*)-TZ2PIQ-A6** prepared by the Mg-mediated reaction; **B)** *anti*-**(*S*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **C)** co-injection of **(*S*)-TZ2PIQ-A6** prepared by the in situ click reaction and *anti*-**(*S*)-TZ2PIQ-A6** prepared by the Cu(I)-catalyzed reaction; **D)** in situ click reaction using purified AChE.

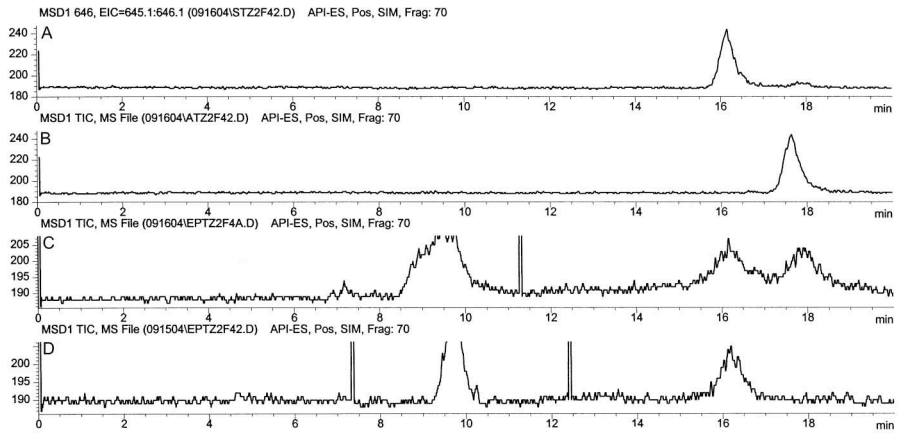
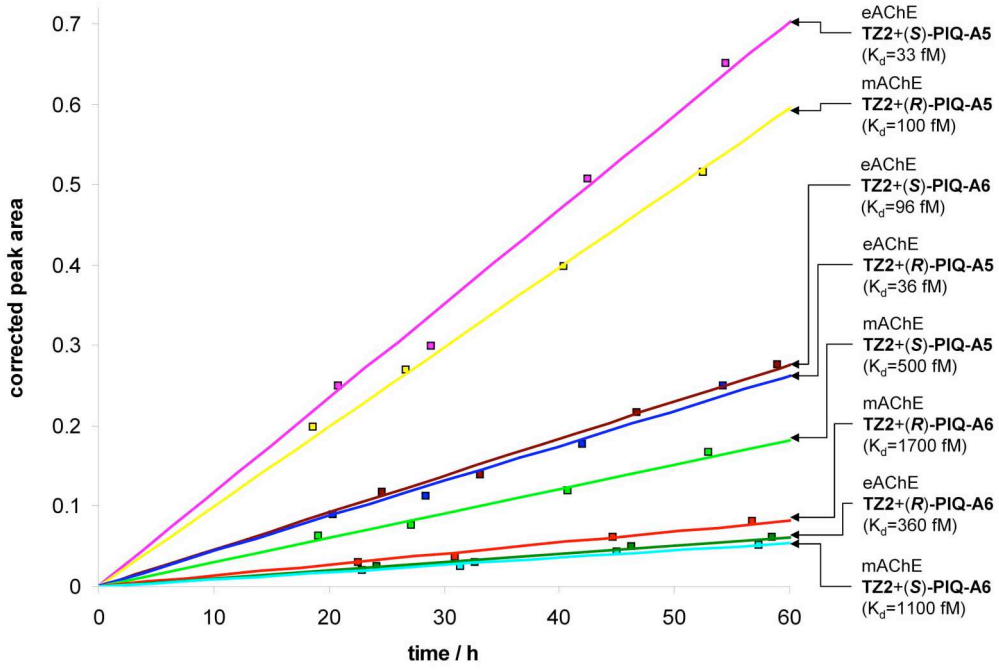


Figure S8. Comparison of the rates of triazole formation between **TZ2** and the enantiomerically pure building blocks: **(*S*)-PIQ-A5**, **(*R*)-PIQ-A5**, **(*S*)-PIQ-A6**, and **(*R*)-PIQ-A6**. All experiments were performed at 37 °C at enzyme concentrations of 1 μM for eel and mouse AChE, at a 20 μM **PIQ-A6**/**PIQ-**

A5 building block concentration, and at a 5 μM **TZ2** concentration. The concentration of the eel enzyme was determined exactly by an Ellman activity test. Interestingly, the comparison of the reaction rates with the corresponding dissociation constants shows no clear correlation between rates of triazole formation and dissociation constants. As a general trend, eel AChE produces triazoles faster with the (*S*)-enatiomer building blocks, while mouse AChE generates triazoles faster with the (*R*)-enantiomer building blocks.

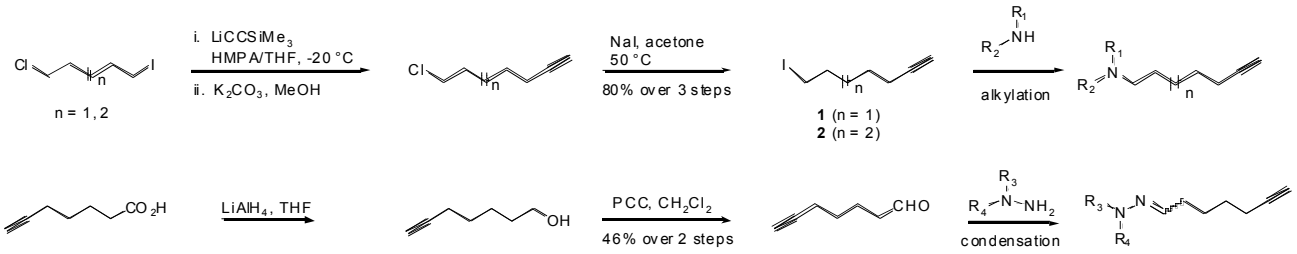


Synthesis of building blocks and triazole inhibitors.

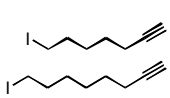
CAUTION! All of the compounds described here (and especially the most potent polyvalent inhibitors) are potentially neurotoxic. They must be handled with extreme care by trained personnel.

The acetylenic building blocks were readily synthesized by alkylating commercially available amines with iodoalkynes **1** and **2** or by forming hydrazones from 7-heptynal (**3**) and in-house available hydrazines. The synthesis of the linker reagents is shown in Scheme 1.

Scheme 1. Synthesis of linker modules.



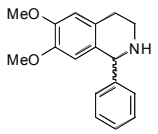
7-Iodohept-1-yne (1) and 8-iodooct-1-yne (2). The iodoalkynes were prepared by a modified literature procedure.[4] A mixture of dry THF (30 mL), HMPA (30 mL) and 1-chloro-5-iodopentane



(9.30 g, 40 mmol, starting material for **1**) or 1-chloro-6-iodohexane (9.86 g, 40 mmol, precursor for **2**) in a dried 500mL flask was cooled to -30°C under an atmosphere of nitrogen. A commercially available 0.5M solution of the lithium salt of trimethylsilyl acetylene in THF (84 mL, 1.05 equiv.) was added to the reaction mixture over a period of 5 minutes. The reaction was allowed to warm to room temperature and monitored by GC. After the starting materials were consumed (approx. 1 hour), saturated aqueous ammonium chloride was added to the solution and the mixture was extracted twice with hexanes (200 mL). The organic layer was washed twice with water (100 mL), followed by brine and dried with anhydrous $MgSO_4$, then evaporated under reduced pressure to afford 93-94% of the crude acetylenes, which were used in the next step without purification.

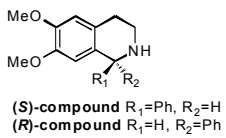
The silyl acetylenes were deprotected by treating solutions in methanol (100 mL) with K_2CO_3 (20g) over night at room temperature to give the corresponding terminal acetylenes in excellent purity (GC).

Water was added (100 mL) to the reaction mixture and the resulting mixture was extracted twice with a 1:1 mixture of diethyl ether and hexanes (200 mL). The organic layer was washed twice with water (100 mL), followed by brine and dried with anhydrous $MgSO_4$, then evaporated under reduced pressure to give the crude acetylenes in 90% yield. The latter were dissolved in acetone (25 mL) and NaI (2 equiv.) was added. The solutions were stirred in sealed tubes at 80°C for 2 days, after which time GC showed complete conversion. The solvent was removed under reduced pressure and the mixture was dissolved in hexanes (100 mL) and filtered. After the evaporation of the solvent the final iodoalkynes **1** and **2** were isolated in 88% yield and used in subsequent alkylation reactions without purification.



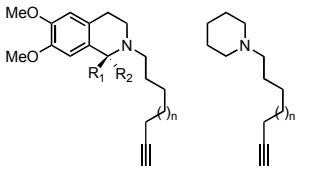
(±)-6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline. A large-scale synthesis of racemic 6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline was performed by following a literature procedure,[5] starting from 3,4-dimethoxyphenethyl amine (53.3 g, 294 mmol) and benzaldehyde (31.2 g, 1 equiv.). A modification was introduced in the work-up procedure, where the crude trifluoroacetate of the amine product was treated with aqueous Na_2CO_3 and extracted with chloroform. After drying with anhydrous $MgSO_4$ and evaporating the solvent under reduced pressure, the solid was treated with 2M aqueous HCl. Most of the water was evaporated and the wet solid was triturated twice with diethyl ether. The ether layer was discarded; the solid was treated with aqueous KOH (2M) and extracted three times with CH_2Cl_2, organic layer was dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The final product was recrystallized from hot ethanol to give the product as colorless crystals (9.02 g, 73% yield). m.p. 112-114°C (lit.[6] 112-113°C); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.34-7.22 (m, 5H), 6.63 (s, 1H), 6.24 (s, 1H), 5.04 (s, 1H), 3.87 (s, 3H), 3.63 (s, 3H), 3.21 (dt, J=12.2 Hz, 5.0 Hz, 1H), 3.08-3.00 (m, 1H), 2.98-2.88 (m, 1H), 2.74 (dt, J=15.6 Hz, 5.0 Hz, 1H),

1.86 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): 147.45, 146.90, 144.81, 129.77, 128.79, 128.29, 127.57, 127.24, 111.26, 110.79, 61.38, 55.74 (2C), 41.79, 29.25.



(*S*)-compound R_1=Ph, R_2=H
(*R*)-compound R_1=H, R_2=Ph

(+)-6,7-dimethoxy-1*R*-phenyl-1,2,3,4-tetrahydroisoquinoline and (-)-6,7-dimethoxy-1*S*-phenyl-1,2,3,4-tetrahydroisoquinoline. The racemate was resolved by following the literature procedure.[6] (±)-6,7-Dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline (9.02 g, 33.5 mmol) was co-crystallized with 2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid monohydrate (10.4 g, 1 equiv.) from 680 mL of hot isopropanol, affording after 24 hours 4.80 g of the salt of the (*S*)-enantiomer-enriched amine (90% d.e.) as a precipitate, which was filtered off. The amine was liberated by stirring the solid with 400 mL of 4M aqueous NaOH and 600 mL of diethyl ether for 1h. The aqueous layer was extracted once with diethyl ether and the combined organic layers were dried with anhydrous $MgSO_4$. After the removal of the solvent under reduced pressure the slightly yellow solid was recrystallized twice from hot ethanol (10-20 mL/g of amine) to afford the (*S)*-enantiomer in excellent purity (1.81 g, 40% of the initial amount, >99.8 e.e., $[\alpha]_D^{23}$=-23.7 (c=0.5, $CHCl_3$); (lit.[6] $[\alpha]_D^{20}$=-20 (c=0.5, $CHCl_3$); m.p. 133.5-135°C (lit.[6] 132°C)). The mother liquor from the recrystallization of the diastereomeric salts was evaporated, transformed into the (*R)*-secondary amine as described above for the (*S*)-enantiomer, and recrystallized twice to give the pure (*R)*-enantiomer (2.21 g, 49% of the initial amount, >99.8 e.e., $[\alpha]_D^{23}$=+23.8 (c=0.5, $CHCl_3$); (lit.[7] $[\alpha]_D^{23}$=+16.3 (84% ee by HPLC c=1.04, $CHCl_3$)). The enantiomeric purity of the samples was determined by HPLC using a Chiralcel OJ column (hexanes:*i*-propanol 2:3, 0.5 mL/min, (*S)*-isomer 11.9 min., (*R)*-isomer 18.3 min., baseline separation). The absolute configuration was assigned using literature information.[6,7]

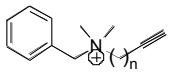


(*S*)-PIQ-A5 n=1, R_1=Ph, R_2=H **PIP-A5** n=1
(*R*)-PIQ-A5 n=1, R_1=H, R_2=Ph **PIP-A6** n=2
(*S*)-PIQ-A6 n=2, R_1=Ph, R_2=H
(*R*)-PIQ-A6 n=2, R_1=H, R_2=Ph
IIQ-A5 n=1, R_1, R_2=H, *i*Pr
IIQ-A6 n=2, R_1, R_2=H, *i*Pr
IQN-A5 n=1, R_1, R_2=H, CH_2CN
IQN-A6 n=2, R_1, R_2=H, CH_2CN
HIQ-A5n=1, R_1=R_2=H
HIQ-A6 n=2, R_1=R_2=H

General procedure for the synthesis of building blocks: (*R*)-PIQ-A5, (*S*)-PIQ-A5, (*R*)-PIQ-A6, (*S*)-PIQ-A6, IQN-A5, IQN-A6, PIP-A5, PIP-A6, HIQ-A5, HIQ-A6, IIQ-A5 and IIQ-A6. These acetylene reagents were obtained by *N*-alkylation of the respective amines with 7-iodohept-1-yne (**1**) or 8-iodooct-1-yne (**2**). The amines (1 mmol) were dissolved/suspended in acetonitrile (10 mL), and the iodoalkynes (1 equiv.) were added, followed by K_2CO_3 (2-3 equiv.) and water (1 mL). The biphasic mixture was stirred vigorously overnight to 3 days at 60°C, then evaporated and purified by chromatography on silica gel using hexane-ethyl acetate as the eluent to afford the pure amines in 57-75% yield.

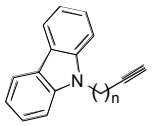
(-)-2-(Hept-6-ynyl)-6,7-dimethoxy-1*R*-phenyl-1,2,3,4-tetrahydroisoquinoline ((*R*)-PIQ-A5), (+)-2-(hept-6-ynyl)-6,7-dimethoxy-1*S*-phenyl-1,2,3,4-tetrahydroisoquinoline ((*S*)-PIQ-A5): ^{1}H NMR ($CDCl_3$, 400 MHz): 7.33-7.22 (m, 5H), 6.61 (s, 1H), 6.19 (s, 1H), 4.49 (s, 1H), 3.86 (s, 3H), 3.61 (s, 3H), 3.15 (dt, J=11.8 Hz, 5.0 Hz, 1H), 3.03-2.92 (m, 1H), 2.78 (dt, J=16.0 Hz, 4.6 Hz, 1H), 2.62-2.47 (m, 2H), 2.31 (dt, J=12.8 Hz, 6.4 Hz, 1H), 2.13 (dt, J=6.8 Hz, 2.6 Hz, 2H), 1.93 (t, J=2.6 Hz, 1H), 1.55-1.25 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): 147.23, 146.89, 144.24, 130.11, 129.48, 128.01, 126.97, 126.87, 111.58, 110.68, 84.59, 68.08, 67.88, 55.73, 55.70, 53.86, 46.74, 28.21, 28.12, 26.34, 26.25, 18.32; m.p. 55-56°C; **(*R*)-PIQ-A5** $[\alpha]_D^{23}$=-91 (c=1.82, MeOH); **(*S*)-PIQ-A5** $[\alpha]_D^{23}$=+92 (c=1.82, MeOH).

(-)-6,7-dimethoxy-2-(oct-7-ynyl)-1*R*-phenyl-1,2,3,4-tetrahydroisoquinoline ((*R*)-PIQ-A6), (+)-6,7-dimethoxy-2-(oct-7-ynyl)-1*S*-phenyl-1,2,3,4-tetrahydroisoquinoline ((*S*)-PIQ-A6): ^{1}H NMR ($CDCl_3$, 400 MHz): 7.34-7.22 (m, 5H), 6.62 (s, 1H), 6.19 (s, 1H), 4.50 (s, 1H), 3.86 (s, 3H), 3.61 (s, 3H), 3.16 (dt, J=11.8 Hz, 5.0 Hz, 1H), 3.04-2.94 (m, 1H), 2.78 (dt, J=16.0 Hz, 4.6 Hz, 1H), 2.62-2.47 (m, 2H), 2.31 (dt, J=12.8 Hz, 6.4 Hz, 1H), 2.14 (dt, J=7.6 Hz, 2.8 Hz, 2H), 1.93 (t, J=2.8 Hz, 1H), 1.55-1.43 (m, 4H), 1.36-1.14 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): 147.20, 146.85, 144.27, 130.11, 129.47, 127.97, 126.92, 126.86, 111.56, 110.66, 84.67, 68.00, 67.85, 55.70, 55.66, 53.94, 46.74, 28.50, 28.36, 28.10, 26.64, 26.56, 18.26; m.p. 76-77°C; **(*R*)-PIQ-A6** $[\alpha]_D^{23}$=-91 (c=1.82, MeOH); **(*S*)-PIQ-A6** $[\alpha]_D^{23}$=+90 (c=1.82, MeOH).

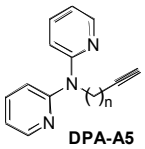


DMB-A5 n=5
DMB-A6 n=6

DMB-A5 and DMB-A6. *N*,*N*-dimethylbenzylamine (2 mmol) was heated neat with 1 equiv. of **1** or **2** at 70°C for 3 hours. The mixture was dissolved in CH_2Cl_2 (5 mL) and the product was precipitated by the addition of pentane (25 mL) and filtered off.

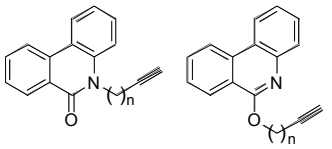


C-A5 n=5
C-A6 n=6

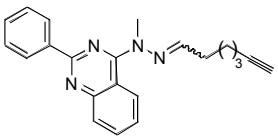


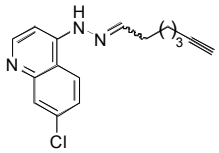
DPA-A5 n=5
DPA-A6 n=6

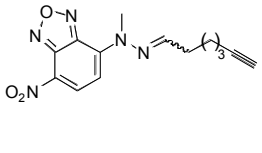
C-A5, C-A6, DPA-A5, DPA-A6. The starting amines (1 mmol) were dissolved in dry THF (5 mL) and sodium hydride (1 equiv.) was added to the solutions, followed by the iodoalkynes (1 equiv.). The solutions were stirred overnight at 70°C and evaporated under reduced pressure. The products were purified by flash chromatography.



PO-A5, PO-A6, PHN-A5, PHN-A6 The O- and N-alkylated compounds were prepared using a modified literature procedure.[8] Potassium *tert*-butoxide (0.275 g, 2.4 mmol) was added to a solution of phenanthridin-6(5H)-one (0.433 g, 2.2 mmol) in DMF (4 mL). After stirring for 10 min, a solution of iodoalkyne **1** or **2** (0.193 g, 0.82 mmol) in DMF (4.0 mL) was added and the mixture was stirred at room temperature overnight. The reaction was quenched with ice water and saturated sodium bicarbonate solution. The precipitate was collected by filtration and washed with dichloromethane into another flask. The filtrate was evaporated. The products were separated and purified by flash chromatography.


PQH-A4


QH-A4

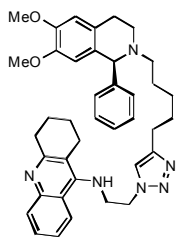

BOH-A4

PQH-A4, QH-A4, BOH-A4 In a scintillation vial, 7-heptynal (0.093 g, 0.84 mmol) and the respective hydrazines (0.40 mmol) were dissolved in 2.0 mL dichloromethane. After stirring at room temperature overnight, the reaction was quenched in 10 mL of water. The reaction mixture was extracted three times with 10 mL portions of ethyl acetate. The combined organic phases were washed with 10 mL of brine, and the solvent was evaporated. The products were separated and purified by flash chromatography.

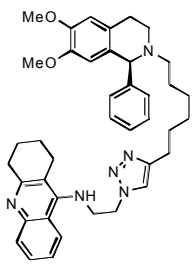
General procedure for the synthesis of *anti*-triazoles.

CAUTION! The compounds described here are potentially neurotoxic and must be handled with extreme care by trained personnel.

Procedures for synthesis of *anti*-triazoles were simplified to minimize manipulations of significant amounts of these compounds; therefore, compounds were not fully characterized. CuI (5 mol-%) was added to a solution of equimolar amounts of the acetylene and the azide (0.125 mmol each) in acetonitrile (0.5 mL) in a nitrogen-purged vial, and the reaction mixture was stirred overnight, whereupon the product precipitated from the solvent. Upon completion of the reaction, THF (0.5 mL) was added to re-dissolve the triazole and the solution was directly deposited onto a flash column in CH_2Cl_2. The polarity of the eluent was gradually increased to eventually reach a CH_2Cl_2-MeOH ratio of 7:3. The product-containing fractions were combined, evaporated and filtered through a plug of basic alumina to remove traces of Cu^{2+} and silica. The product was eluted with $CHCl_3$ –> $CHCl_3$-MeOH (9:1), and the product-containing fractions were combined and evaporated to yield the triazoles in 80-90% yields.



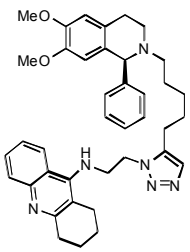
***anti*-(*S*)-TZ2PIQ-A5:** ^{1}H NMR ($CDCl_3$, 400 MHz): 7.90 (d, J=8.2 Hz, 1H), 7.73 (d, J=8.2 Hz, 1H), 7.54-7.49 (m, 1H), 7.33-7.28 (m, 1H), 7.26-7.13 (m, 5H), 7.10 (s, 1H), 6.55 (s, 1H), 6.12 (s, 1H), 4.66-4.59 (m, 1H), 4.46-4.41 (m, 3H), 3.98-3.92 (m, 2H), 3.80 (s, 3H), 3.55 (s, 3H), 3.12-2.99 (m, 3H), 2.95-2.86 (m, 1H), 2.72 (dt, J=16.0 Hz, 4.6 Hz, 1H), 2.63-2.40 (m, 6H), 2.30-2.22 (m, 1H), 1.90-1.76 (m, 4H), 1.55-1.42 (m, 4H), 1.32-1.14 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): 158.26, 149.35, 148.44, 147.12, 146.75, 146.52, 144.02, 129.89, 129.36, 128.50, 128.14, 127.86, 126.81, 126.72, 124.19, 122.06, 121.40, 120.13, 117.46, 111.46, 110.58, 67.68, 55.60, 55.57, 53.74, 50.20, 47.71, 46.51, 33.52, 28.97, 27.93, 26.65, 26.32, 25.34, 24.62, 22.66, 22.40.



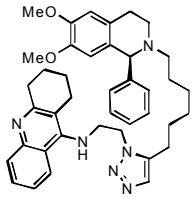
***anti*-(*S*)-TZ2PIQ-A6:** ^{1}H NMR ($CDCl_3$, 400 MHz): 7.89 (d, J=8.2 Hz, 1H), 7.73 (d, J=8.2 Hz, 1H), 7.56-7.50 (m, 1H), 7.35-7.30 (m, 1H), 7.29-7.18 (m, 5H), 7.11 (s, 1H), 6.58 (s, 1H), 6.15 (s, 1H), 4.50 (m, 4H), 3.97-3.91 (m, 2H), 3.83 (s, 3H), 3.58 (s, 3H), 3.15-3.08 (m, 1H), 3.03 (t, J=6.4 Hz, 2H), 2.99-2.90 (m, 1H), 2.74 (dt, J=16.0 Hz, 4.4 Hz, 1H), 2.65-2.43 (m, 6H), 2.30-2.22 (m, 1H), 1.92-1.78 (m, 4H), 1.60-1.51 (m, 2H), 1.50-1.41 (m, 2H), 1.28-1.12 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): 158.83, 148.88, 148.63, 147.23, 147.17, 146.81, 144.21, 130.05, 129.43, 128.89, 128.30, 127.92, 126.86, 126.82, 124.20, 121.93, 121.32, 120.52, 117.99, 111.54, 110.63, 67.80, 55.67, 55.65, 53.92, 50.34, 47.77, 46.64, 33.97, 29.27, 28.95, 28.05, 26.88, 26.57, 25.44, 24.73, 22.83, 22.66.

General procedure for the synthesis of *syn*-triazoles. (Caution! These compounds are potentially toxic and dangerous; all manipulations must be performed with extreme care!). Procedures for synthesis of *syn*-triazoles were simplified to minimize manipulations of significant amounts of these compounds; therefore, compounds were not fully characterized. EtMgCl in THF (2.9 M solution, 1 equiv.) was added to a solution of the acetylene (1 mmol) in dry THF (1.5 mL) at room temperature under a nitrogen atmosphere. The solution was heated to 60°C for 30 min., cooled down to room temperature, transferred to a dry flask containing neat **TZ2** (0.48 mmol) and stirred to dissolve the solid. The solution was heated to 60°C for 4 hours, then cooled down to room temperature and quenched with aqueous ammonium chloride, followed by 5% NaOH until the aqueous layer became slightly cloudy. The organic layer was directly deposited onto a flash column in CH_2Cl_2; the aqueous layer was additionally stirred with 1 mL of THF, which after separation was deposited onto the same column. Flash chromatography was performed and the polarity of the eluent was gradually increased to reach a

CH_2Cl_2-MeOH ratio of 7:3. The product-containing fractions were combined, evaporated and filtered through a plug of basic alumina to remove silica. The product was eluted with $CHCl_3$ –> $CHCl_3$-MeOH (9:1), and the product-containing fractions were combined and evaporated to yield the triazoles in 65-80% yields.



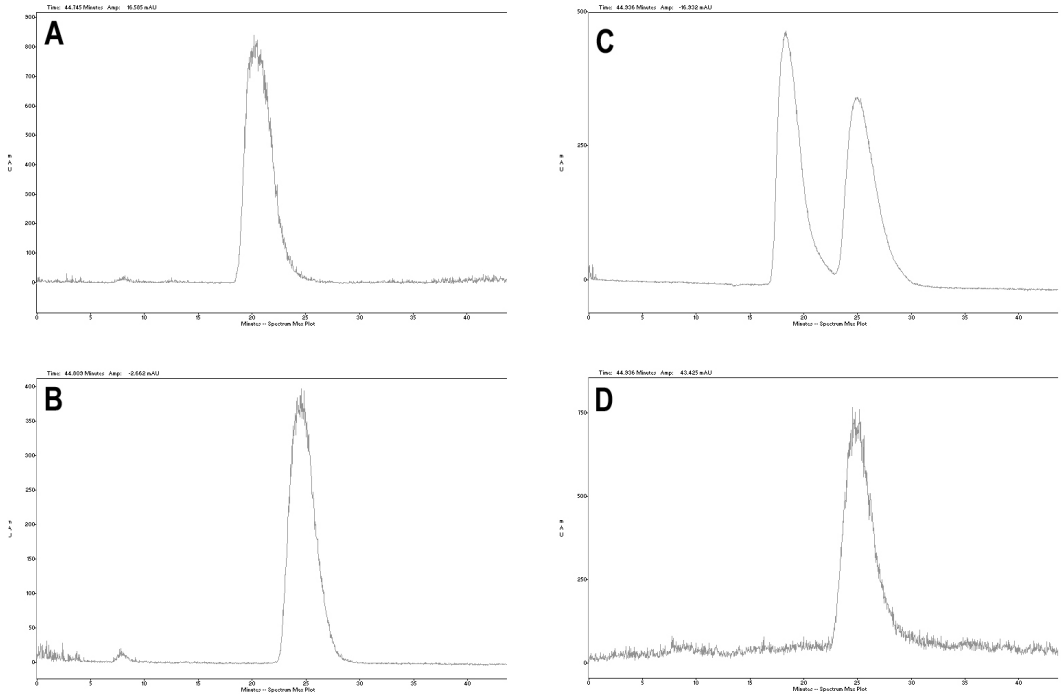
***syn*-(*S*)-TZ2PIQ-A5:** ^{1}H NMR ($CDCl_3$, 400 MHz): 7.99 (d, J=8.4 Hz, 1H), 7.84 (d, J=8.4 Hz, 1H), 7.55-7.50 (m, 1H), 7.39 (s, 1H), 7.35-7.30 (m, 1H), 7.27-7.16 (m, 5H), 6.58 (s, 1H), 6.13 (s, 1H), 5.37 (bs, 1H), 4.42 (s, 1H), 4.38-4.33 (m, 2H), 4.14-4.07 (m, 2H), 3.83 (s, 3H), 3.57 (s, 3H), 3.15-3.02 (m, 3H), 3.00-2.91 (m, 1H), 2.74 (dt, J=16.0 Hz, 4.2 Hz, 1H), 2.62-2.42 (m, 4H), 2.34 (t, J=7.8 Hz, 2H), 2.24 (ddd, J=12.8 Hz, 8.0 Hz, 5.2 Hz, 1H), 1.89-1.76 (m, 4H), 1.52-1.30 (m, 4H), 1.28-1.10 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): 158.65, 148.96, 147.18, 147.01, 146.84, 144.20, 137.66, 132.16, 130.00, 129.42, 128.70, 128.36, 127.93, 126.96, 126.69, 124.21, 121.92, 120.45, 117.96, 111.48, 110.58, 68.14, 55.66, 55.62, 53.47, 47.41, 46.89, 46.83, 33.82, 28.20, 27.41, 26.43, 26.22, 24.67, 22.80, 22.75, 22.61.



***syn*-(*S*)-TZ2PIQ-A6:** ^{1}H NMR ($CDCl_3$, 400 MHz): 7.89 (d, J=7.8 Hz, 1H), 7.74 (d, J=7.8 Hz, 1H), 7.52 (ddd, J=8.4 Hz, 6.8 Hz, 1.6 Hz, 1H), 7.43 (s, 1H), 7.32 (ddd, J=8.4 Hz, 6.8 Hz, 1.6 Hz, 1H), 7.28-7.17 (m, 5H), 6.58 (s, 1H), 6.14 (s, 1H), 4.67 (bt, J=7.2 Hz, 1H), 4.46-4.42 (m, 1H), 4.28-4.23 (m, 2H), 4.05-3.98 (m, 2H), 3.83 (s, 3H), 3.58 (s, 3H), 3.11 (dt, J=11.6 Hz, 5.2 Hz, 1H), 3.03 (t, J=6.4 Hz, 2H), 3.01-2.90 (m, 1H), 2.74 (dt, J=16.0 Hz, 4.4 Hz, 1H), 2.62-2.42 (m, 4H), 2.30-2.20 (m, 3H), 1.92-1.78 (m, 4H), 1.48-1.35 (m, 4H), 1.28-1.06 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): 158.68, 148.76, 147.09, 147.07, 146.72, 144.13, 137.62, 132.02, 129.91, 129.33, 128.75, 128.17, 127.82, 126.78, 126.65, 124.08, 121.81, 120.43, 117.95, 111.41, 110.52, 67.84,

55.53, 55.56, 53.65, 47.32, 46.82, 46.65, 33.85, 28.58, 28.02, 27.59, 26.47, 26.38, 24.59, 22.72, 22.61, 22.56.

Figure S9. Test of potential racemization of **(*R*)-PIQ-A5** in the Mg-mediated triazole formation by recovery of unreacted acetylene **(*R*)-PIQ-A5** from the reaction mixture followed by confirmation of enantiopurity by chiral HPLC. HPLC traces of **A) (*S*)-PIQ-A5**; **B) (*R*)-PIQ-A5**; **C)** racemic mixture of **(*S*)-PIQ-A5** and **(*R*)-PIQ-A5**; and **D) (*R*)-PIQ-A5** recovered from the Mg-mediated *syn*-triazole formation. Comparison of the HPLC traces revealed that the enantiopure building block **(*R*)-PIQ-A5** did not racemize in the Mg-mediated triazole formation. Since there was no epimerization of the excess **(*R*)-PIQ-A5** observed, it is assumed that in the triazole formation, no epimerization of the final triazoles occurred. The chromatography was performed on a Chiralcel OJ column (hexanes:*i*-propanol 95:5, 0.4 mL/min).



Determination of AChE - inhibitor association and dissociation rate constants. All kinetic parameters of inhibitor binding to and dissociation from eel and mouse AChE were measured as described previously except for one modification. In determinations of the first order dissociation rate constants instead of DNA, purified inactive mouse AChE mutant Ser203Ala (20 – 70 nM) was used to sequester inhibitor upon its release from the complex with the active wild type AChE , and prevent its re-association with wild type AChE when inhibitor concentrations in the reactivation medium are above their K_d. This modification was necessary since phenyltetrahydroisoquinoline derivatives do not intercalate with DNA as efficiently as phenylphenanthridinium derivatives, while all of them bind to Ser203Ala mutant with similar potency as to wild type AChE.

All experiments were performed in at least triplicate with the standard error of determination smaller than 20% of the mean value. The measurements were performed in 0.1 M phosphate buffer pH 7.0 at 22 °C on a SX.18 MV stopped-flow instrument (Applied Photophysics) or Cary 1E UV/VIS spectrophotometer (Varian).

Table S1. Free energies of binding to AChE

Inhibitor		$-RTlnK_d$ (kcal mol^{-1})	AChE source
***(S)*-TZ2PIQ-A5**	*syn-*	18.2	eel
		16.6	mouse
	anti-	13.1	eel
		12.2	mouse
***(R)*-TZ2PIQ-A5**	*syn-*	18.2	eel
		17.6	mouse
	anti-	13.1	eel
		13.2	mouse
***(S)*-TZ2PIQ-A6**	*syn-*	17.6	eel
		16.1	mouse
	anti-	13.1	eel
		12.7	mouse
***(R)*-TZ2PIQ-A6**	*syn-*	16.8	eel
		15.9	mouse
	anti-	13.2	eel
		13.0	mouse
TZ2HIQ-A6	*syn-*	16.1	eel
		15.2	mouse
	anti-	11.7	eel
		11.8	mouse

Table S2. Energy increments for *syn*- to *anti*- isomerisation.

Δ Inhibitor ***syn → anti***	**Δ (-RT*ln*K_d)** (kcal mol^{-1})	AChE source
(*S*)-TZ2PIQ-A5	5.1 4.4	eel mouse
(*R*)-TZ2PIQ-A5	5.0 4.4	eel mouse
(*S*)-TZ2PIQ-A6	4.5 3.5	eel mouse
(*R*)-TZ2PIQ-A6	3.6 2.9	eel mouse
TZ2HIQ-A6	4.4 3.4	eel mouse
mean ± SD	4.5 ± 0.6 3.7 ± 0.7	eel mouse
mean ± SD : S	4.4 ± 0.7	ALL
mean ± SD : R	4.0 ± 0.9	ALL
mean ± SD : A5	4.7 ± 0.4	ALL
mean ± SD : A6	3.7 ± 0.6	ALL
mean ± SD	*4.1 ± 0.8*	*ALL*

Table S3. Energy increments for linker length reduction.

Δ Inhibitor **A6 → A5**		**Δ (-RT*ln*K$_d$)** (kcal mol^{-1})	AChE source
(*S*)-TZ2PIQ	*syn-*	- 0.63 - 0.46	eel mouse
	anti-	0.030 0.43	eel mouse
(*R*)-TZ2PIQ	*syn-*	- 1.4 - 1.7	eel mouse
	anti-	0.07 - 0.12	eel mouse
mean ± SD	all all	- 0.45 ± 0.88 - 0.47 ± 0.67	eel mouse
mean ± SD	*syn-* *anti-*	- 1.0 ± 0.6 0.10 ± 0.23	ALL ALL
mean ± SD	*S* *R*	- 0.15 ± 0.48 - 0.77 ± 0.87	ALL ALL
	all	*- 0.47 ± 0.74*	*ALL*

Table S4. Energy increments for inversion of stereochemistry.

Δ Inhibitor **S → R**		**Δ (-RT*ln*K$_d$)** (kcal mol^{-1})	AChE source
TZ2PIQ-A5	*syn-*	0.05 - 0.94	eel mouse
	anti-	- 0.06 - 0.92	eel mouse
TZ2PIQ-A6	*syn-*	0.77 0.26	eel mouse
	anti-	- 0.10 - 0.38	eel mouse
mean ± SD	all all	0.17 ± 0.41 - 0.50 ± 0.57	eel mouse
mean ± SD	*syn-* *anti-*	0.034 ± 0.72 - 0.36 ± 0.40	ALL ALL
mean ± SD	A5 A6	- 0.47 ± 0.54 0.14 ± 0.50	ALL ALL
	all	*- 0.16 ± 0.58*	*ALL*

Table S5. Energy increments for structural changes.[#]

Δ Inhibitor	**mean Δ (-RT*ln*K$_d$)** (kcal mol^{-1})	AChE source
syn*→ *anti	4.5 ± 0.6 3.7 ± 0.7	eel mouse
A5→ A6	0.45 ± 0.88 0.47 ± 0.67	eel mouse
S→ R	0.17 ± 0.41 - 0.50 ± 0.57	eel mouse
Σ Δ(-RT*ln*K$_d$)	**5.1** *(5.1)* **3.7** *(3.7)*	eel mouse

[#] The numbers outside the parentheses are obtained by summation of individual energy increments from the same table. The numbers in parentheses are obtained by direct subtraction of the ΔG values of the two compounds representing two extremes.

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