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Evaluation of Short-tether Bis-THA AChE Inhibitors. A Further Test of the Dual Binding Site Hypothesis

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Abstract—To provide a further test of the dual binding site hypothesis proposed for acetylcholinesterase (AChE) inhibitor heptylene-linked bis-(9-amino-1,2,3,4-tetrahydroacridine) **A7A**, short-tether (ethylene–hexylene) homologs **A2A**–**A6A** were prepared. En route to these compounds, convenient and scaleable syntheses of useful pharmaceutical intermediate 9-chloro-1,2,3,4-tetrahydroacridine **3** and **A7A** were developed. AChE and butyrylcholinesterase (BChE) inhibition assays of **A2A**–**A10A** confirm that a seven methylene tether (**A7A**) optimizes AChE inhibition potency and AChE/BChE selectivity. Finally, these studies indicate that simultaneous binding of alkylene-linked 9-amino-1,2,3,4-tetrahydroacridine dimers to the catalytic and peripheral sites of AChE is possible with a tether length as short as 5 methylenes.

Introduction

9-Amino-1,2,3,4-tetrahydroacridine (THA) dimer **A7A** is a promising drug candidate for the palliative treatment of Alzheimer's disease, due to its remarkable enhancement in acetylcholinesterase (AChE) inhibition potency and AChE/butyrylcholinesterase (BChE) selectivity relative to THA itself (Table 1).[1] The synthesis and evaluation of **A7A** was predicated on computational studies which indicated affinity of THA to both the catalytic site of AChE and to aromatic amino acid residues Trp^{279}, Tyr^{70}, Phe^{290} (the 'peripheral site') near the mouth of the active site gorge.[2] A series of four homologous THA dimers (**AnA**) was screened, which showed a monotonic increase in AChE inhibition potency and AChE/BChE selectivity from **A10A** to **A7A** (Table 1).

A series of THA–toluene heterodimers (**AnT**, n = 3–9) was also evaluated, which indicated that a spacing of 7 methylene units optimized both potency and selectivity (Table 2).

Based on the **AnT** results, it was predicted that heptylene-linked bis-THA (**A7A**) is the optimum THA dimer.[1] However to date, this conjecture remains *unproven*. Demonstration that **A7A** is superior to homologous 'short-tether' THA dimers (i.e. **AnA**, n < 7) is important not only from the perspective of drug development, but as a further physical test of the dual binding site hypothesis. If simultaneous binding to the catalytic and peripheral sites is responsible for the enhanced AChE inhibition potency of **A7A**, there should be a well-defined minimum tether length, beyond which dual site binding is impossible and AChE IC_{50} rises dramatically.

Chemical synthesis

Application of the previously described THA alkylation protocol[3] to the synthesis of desired homologs **A2A**-**A6A** was not successful. In the case of **A6A** the desired product could be obtained in low yield (37%), but was accompanied by significant amounts of the intramolecular cyclization product **1**[n = 6] and elimination product **2**[n = 6] (Scheme 1).

Attempts to increase the yield of the **A6A** product by variation of the base, reaction temperature, concentration and solvent were fruitless. For n = 3–5, the cyclization and elimination pathways completely overwhelmed the desired bimolecular substitution reaction, to the point that none of the desired **AnA** products could be obtained.

To overcome these difficulties we explored reaction of 9-chloro-1,2,3,4-tetrahydroacridine **3** with 1,n-diamines. Similar strategies have been successfully applied to the

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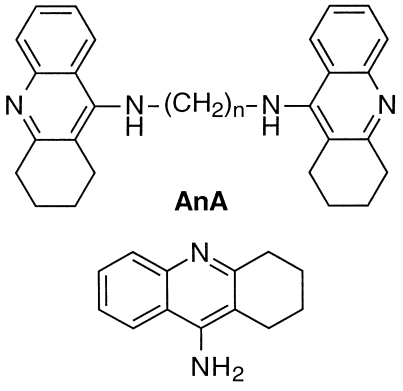
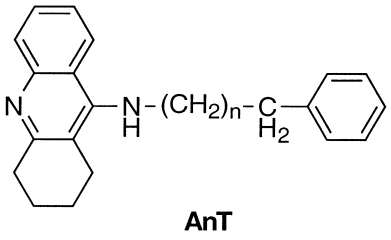
Table 1. AChE inhibition potency and selectivity of **A7A**–**A10A** and THA[a]



n	AChE IC_{50} (nM)	Selectivity for AChE[b]
7	0.40 ± 0.025	980
8	0.66 ± 0.20	520
9	0.77 ± 0.11	250
10	3.1 ± 0.75	140
THA	590 ± 37	0.1

[a]Data taken from reference 1.
[b]Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE).

Table 2. AChE inhibition potency and selectivity of THA–toluene heterodimers **AnT**[a]

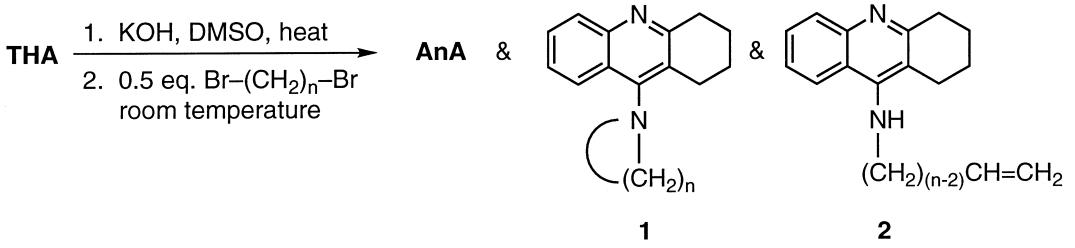


n	AChE IC_{50} (nM)	Selectivity for AChE[b]
3	1,400 ± 140	nd
4	1,500 ± 190	0.4
5	610 ± 23	1.4
6	390 ± 12	2.6
7	210 ± 19	11.4
8	20,000 ± 950	0.5
9	27,000 ± 4,000	0.4

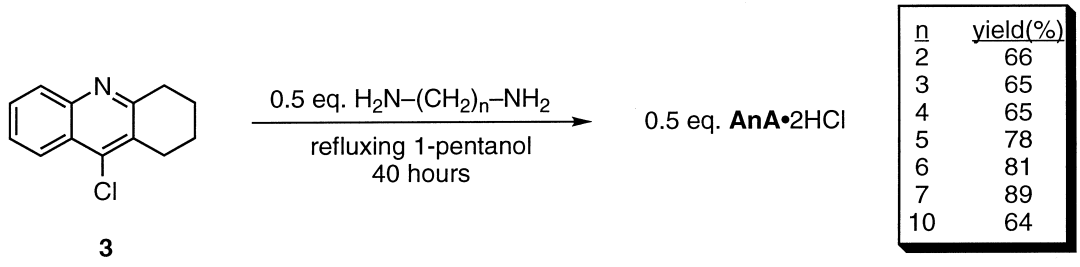
[a]Data taken from reference 1.
[b]Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE).

synthesis of alkylene-linked bis-9-aminoacridines[4] and bis-4-aminoquinolines,[5] and **3** has been previously suggested[3] as a possible starting material for **A7A**. Reaction of **3** and 1,7-heptanediamine in the absence of solvent proceeded to low conversion, despite the application of mild pressure (150 °C, sealed tube). Non-polar, dipolar aprotic, and protic solvents were then explored, at atmospheric pressure and in sealed tube reactions. The optimum system proved to be refluxing 1-pentanol[6] at atmospheric pressure for 40 h, which provided a sufficiently high temperature, gave excellent conversion, and facilitated direct crystallization of pure **A7A**·2HCl (> 98 area% by HPLC) from the reaction mixture in 89% yield during aqueous workup (Scheme 2).

This amination procedure is easily carried out on a large scale, in contrast to the previously reported synthesis. Synthesis of **A7A** by alkylation of THA[3] occurs in high yield (87%), but requires a careful chromatographic purification step to remove both residual **THA** and cyclization product **1**[n = 7].[7] We have confirmed that unless these materials are removed by chromatography, neither **A7A** nor **A7A**·2HCl can be isolated by crystallization. This constraint renders scale-up of the alkylation reaction difficult.



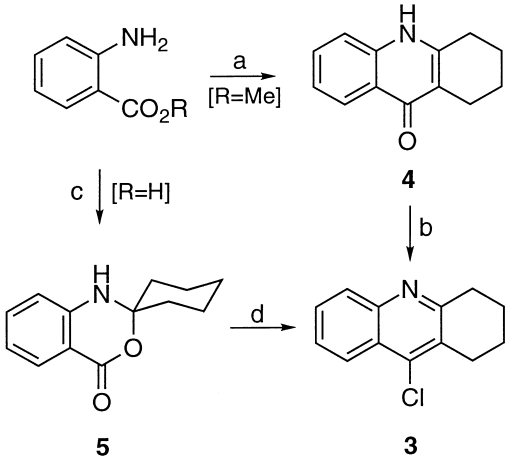
Scheme 1. Synthesis of THA dimers **AnA** by alkylation of THA.



Scheme 2. Synthesis of THA dimers **AnA** by amination of **3**.

The amination strategy was then applied to synthesis of the desired short-tether homologs. Moderate to good yields were obtained; bis-hydrochloride salts **AnA**·2HCl precipitated directly from the reaction mixture upon cooling, or upon addition of aqueous HCl. In the course of developing the protocol described above, we also developed an improved synthesis of **3**. Previously reported syntheses of **3** proceed via chlorination of the corresponding 1,2,3,4-tetrahydro-9-acridone **4**, typically in 80–85% yield. Compound **4** is commercially available, but is moderately expensive. The most practical synthesis of **4** reported thus far is a modified von Niementoski reaction of methyl anthranilate and cyclohexanone, which proceeds in only 50% yield.[8,9] We have found that isolation of the von Niementoski product **4** is not necessary; under milder conditions (toluene reflux) anthranilic acid and cyclohexanone provide spiro-carbinolamine ester **5**[10] in 93% yield (Scheme 3).

Direct treatment of **5** with 4 equivalents of phosphorus oxychloride provided **3** in high yield (96%), for an overall yield of 89%. This procedure has been carried out on up to a 60 g scale. Because **3** has also proven useful in the synthesis of endothelin-A receptor agonists,[11] and chemo- and radiosensitizers for cancer therapy,[12] we believe the convenient procedure outlined above will facilitate further exploration of medicinal applications of the 1,2,3,4-tetrahydroacridin-9-yl unit.



Scheme 3. Improved Synthesis of **3**. a) 1.2 equiv. cyclohexanone, 2.0 equiv. P_2O_5, 2.0 equiv. *N,N*-dimethylcyclohexylamine, 170 °C (50%). b) 10 equiv. $POCl_3$, reflux (85%). c) 1.2 equiv. cyclohexanone, toluene, Dean–Stark Trap, reflux (93%). d) 4 equiv. $POCl_3$, reflux (96%).

AChE and BChE assay results and discussion

Drugs **A2A**·2HCl–**A10A**·2HCl possessed adequate aqueous solubility (≥10 mg/mL) and were assayed for AChE (rat cortex) and BChE (rat serum) inhibition potency (Ellman method[13]) without the need for the ethanol cosolvent used in the previous study of **A7A**. The IC_{50} values obtained for THA and **A7A–A10A** differ somewhat from that previously obtained, although the same trends are observed: **A7A** is optimum and considerably more potent and selective than THA (cf. Tables 1 and 3). To provide an additional check, enzyme kinetic studies were performed in rat cortex for **A7A** and THA. Both drugs exhibited mixed inhibition, and the rat cortex K_I values (**A7A** 2.6 nM, THA 104 nM) matched the previously determined human cerebellum K_I values (**A7A** 1.4 nM, THA 80 nM)[1] rather closely.

Review of the data for the complete series of **AnA** dimers reveals that as tether length is decreased below 7 methylenes, performance deteriorates (Table 3).

Thus, as anticipated, a tether of 7 methylene units is indeed optimum for both AChE inhibition potency and selectivity. Interestingly, new short-tether THA dimers **A5A** and **A6A** still display considerable enhancement in AChE inhibition potency relative to THA itself (Table 3), a result which suggests that simultaneous dual site binding can still occur at these shorter tether lengths. However, as the tether length decreases further ($n < 5$), the AChE IC_{50} increases rapidly (Fig. 1). **A4A** and **A3A** have nearly equivalent potency to THA, suggesting that simultaneous dual site binding is no longer possible. **A2A** is considerably less potent than THA, possibly as a consequence of destabilizing non-bonded interactions at the bottom of the narrow active site gorge of AChE.

Thus the anticipated dramatic increase in AChE IC_{50} with decreasing tether length is indeed observed.

In contrast, inspection of the BChE data for **A2A** to **A10A** reveals no clear trend in inhibition potency (Table 3, Fig. 1). Whereas AChE IC_{50} values of **A2A-A10A** span a 500-fold range, BChE IC_{50} values vary over only a 3-fold range and at best (**A2A, A8A**) only equal that of THA itself. These observations are consistent with the lack of a 'peripheral site' in BChE, engendered by the substitution of nonaromatic residues (Ala^{279}, Asn^{70}, Gln^{121}) in BChE at positions corresponding to the peripheral site of AChE. The fact that BChE IC_{50} values do not detectably increase at the shortest tether lengths ($n = 2–3$) suggests that BChE is better able than AChE to accomodate steric bulk near the catalytic site. Such a result is consistent with the fact

Table 3. AChE and BChE inhibitory potency of THA dimers **AnA**·2HCl and **THA**·HCl

Drug	AChE IC_{50} (nM)[a]	BChE IC_{50} (nM)[b]	Selectivity for AChE[c]
A2A·2HCl	711 ± 25	102 ± 4	0.14
A3A·2HCl	254 ± 55	152 ± 17	0.60
A4A·2HCl	157 ± 23	252 ± 9	1.60
A5A·2HCl	28 ± 5	329 ± 21	11.7
A6A·2HCl	3.8 ± 0.4	119 ± 6	31.6
A7A·2HCl	1.5 ± 0.3	149 ± 23	99.4
A8A·2HCl	7.8 ± 0.9	105 ± 13	13.5
A9A·2HCl	31 ± 3	155 ± 25	4.9
A10A·2HCl	40 ± 6	167 ± 12	4.2
THA·HCl	223 ± 11	92 ± 2	0.4

[a]Assay performed using rat cortex homogenate, in the presence of ethopropazine as a specific BChE inhibitor.
[b]Assay performed using rat serum, in the presence of BW284c51 as a specific AChE inhibitor.
[c]Selectivity for AChE is defined as IC_{50}(BChE)/IC_{50}(AChE).

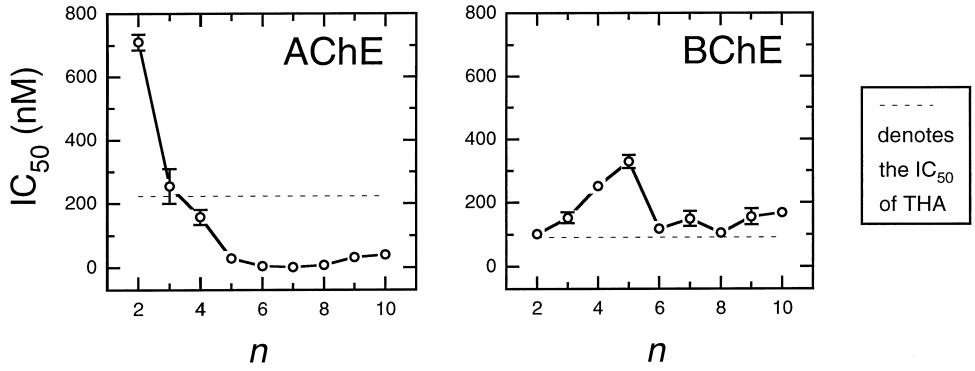


Figure 1. AChE and BChE inhibition by THA dimers **AnA** as a function of tether length n (n = number of methylene units, IC_{50} values from Table 3). Where no error bars are shown, the error is smaller than the symbol.

that BChE requires a larger active site gorge to accomodate its natural substrate BCh.[14]

Conclusion

This study has provided greatly improved and convenient syntheses of 9-chloro-1,2,3,4-tetrahydroacridine **3** and heptylene-linked bis-THA **A7A**. Short-tether homologs **A2A**–**A6A** have been prepared for the first time, and AChE and BChE inhibition studies demonstrate that a tether of 7 methylene units indeed optimizes both AChE inhibition potency and selectivity, consistent with earlier projections. Finally, when the tether is too short (< 5 methylenes) simultaneous binding to both the catalytic and peripheral sites of AChE ('dual-site binding') appears to be impossible.

Experimental

THA was obtained from Sigma. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. Chemical ionization (CI) mass spectra were acquired using CH_4 as the reagent gas. Elemental analysis was performed by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (Shanghai, P.R.C.). Melting points were determined with a Electrothermal 9100 melting point apparatus and are uncorrected. HPLC analysis was performed on a Lichrosorb® RP Select B column (0.4×25 cm, 5 μm), detection at 245 nm, flow rate 1.0 mL/min, with gradient elution. Elution program: 90% A at 0 min, 10% A at 30 min (Buffer A = 0.1% (v/v) aqueous CF_3CO_2H, Buffer B = 1/4 (v/v) Buffer A/CH_3CN).

Spiro[2*H*-3,1-benzoxazine-2,1′-cyclohexan]-4(1*H*)-one (5). Anthranilic acid (50.0 g, 364 mmol) was dissolved in 100 ml of toluene, and combined with cyclohexanone (45.3 ml, 437 mmol). The mixture was heated to reflux (Dean–Stark Trap) until one equivalent of water (6.8 mL) was removed (2 h). Short needle-like crystals formed on cooling to room temperature. The mixture was further cooled to 0 °C, the solid collected by filtration, washed with toluene (50 mL) and ethanol (50 mL), and dried in vacuo, affording 73.2 g (93%) of **5** as white crystals, mp 143.4–145 °C (lit. 155 °C[10]). ^1H NMR and IR data were consistent with the literature.[10]

^1H NMR ($CDCl_3$): δ 1.45–1.55 (m, 4H), 1.78–1.81 (m, 4H), 2.08–2.12 (m, 2H), 4.71 (s, 1H), 6.76 (apparent d, 1H, J = 8.3), 6.90 (apparent t, 1H, 7.5 Hz),7.39 (ddd, 1H, *J* = 1.5, 7.3, 8.1 Hz), 7.906 (dd, 1H, *J* = 1.3, 7.9 Hz); ^{13}C NMR ($CDCl_3$): 22.02, 24.81, 35.59, 90.14, 113.38, 116.26, 119.86, 130.16, 135.28, 145.26, 163.85; MS (CI^+): 218 (M + 1) IR: 3290, 2942, 1682, 1488 cm^{-1}

9-Chloro-1,2,3,4-tetrahydroacridine (3). Spiro-carbinolamine ester **5** (67.0 g, 309 mmol) was added in portions to phosphorus oxychloride (120 mL, 1.29 mol) (*caution*: exotherm). The mixture was heated to reflux (oil bath temperature 120 °C) for 2 h. After cooling to room temperature the reaction was cautiously added dropwise to KOH (540 g) in ice water (1000 mL), with rapid stirring. After the addition was complete a yellow solid had precipitated and the supernatant pH was 10–11. Methylene chloride (1500 mL) was added to dissolve the solid, and the aqueous layer was extracted with CH_2Cl_2 (3×1000 mL). The combined organic extracts were dried ($MgSO_4$), filtered and the solvent removed in vacuo to provide 64.0 g (96%) of **3** as a yellow solid, mp 66.5–67.7 °C (lit. 65–67 °C).[15] ^1H NMR data matched the literature.[15] HPLC indicated 100.0 area % purity (retention time 20.6 min).

^1H NMR ($CDCl_3$): δ 1.91–2.01 (m, 4H), 3.03 (t, 2H, *J* = 5.8 Hz), 3.14 (t, 2H, *J* = 5.6 Hz), 7.55 (ddd, 1H, *J* = 1.2, 7.0, 8.3 Hz), 7.68 (ddd, 1H, *J* = 1.4, 6.9, 8.4 Hz), 7.99 (apparent d, 1H, *J* = 8.4 Hz), 8.17 (dd, 1H, *J* = 1.00, 8.4 Hz); ^{13}C NMR ($CDCl_3$): δ 22.57, 22.61, 27.45, 34.13, 123.63, 125.33, 126.41, 128.57, 128.81, 129.19, 141.39, 146.62, 159.45; MS (CI^+): 218 (M + 1).

Heptylene-linked bis-THA bis-HCl salt (A7A·2HCl). 3 (5.0 g, 23.1 mmol) and 1,7-diaminoheptane (1.5 g, 11.5 mmol) were dissolved in 30 mL of 1-pentanol, and heated to reflux (oil bath temperature 160 °C) for 40 h. After cooling to room temperature, the mixture was poured into a 1-L beaker, and 2N HCl (600 mL) was added to it to precipitate the product. The solid was collected by suction filtration, washed with toluene

(50 mL) and dried in vacuo to afford 5.82 g (89%) of **A7A**·2HCl as a white solid (mp 107–109.4 °C), which was pure by ^{1}H NMR spectroscopy except for trace residual toluene (< 0.8 wt%). HPLC analysis revealed 98.2 area% purity (retention time 24.2 min).

Recrystallization from warm 10% (v/v) aqueous MeOH (50 mL) afforded 4.72 g (72%) of **A7A**·2HCl, increasing the HPLC area% purity slightly (to 99.2%) and the melting point dramatically (mp 145.7–148.1 °C). *Large Scale Synthesis*: **3** (25.0 g, 115 mmol) and 1,7-diaminoheptane (7.50 g, 57.6 mmol) were reacted as above, giving after recrystallization 21.7 g (67%) of **A7A**·2HCl (mp 148.3–150 °C, 99.8 area% purity by HPLC, elemental analysis (below) matches dihydrate).

^{1}H NMR (D_2O): δ 1.38 (s, br, 6H), 1.66–1.79 (m, 12H), 2.34 (s, br, 4H), 2.71 (s, br, 4H), 3.61 (t, 4H, J = 6.8 Hz), 7.22 (apparent t, 2H, J = 7.8 Hz), 7.30 (apparent d, 2H, J = 8.4 Hz), 7.56 (apparent t, 2H, J = 7.7 Hz), 7.80 (apparent d, 2H, J = 8.7 Hz) [HOD peak as reference at 4.8 ppm.]; ^{13}C NMR (CD_3OD): δ 22.67, 23.81, 25.74, 28.44, 30.14, 30.75, 32.29, 49.90, 113.65, 117.85, 120.96, 127.14, 127.31, 134.90, 140.61, 152.52, 158.76; MS (CI^+): Calc. for $C_{33}H_{41}N_4$ (M + 1): 493. Found: 493. Elemental Analysis: Calc. for $C_{33}H_{42}N_4Cl_2·2H_2O$: C, 65.88; H, 7.71; N, 9.31; Cl, 11.78. Found: C, 66.03; H, 7.88; N, 9.31; Cl, 11.80.

Ethylene-linked bis-THA bis-hydrochloride (A2A·2HCl). Ethylene diamine (153 mg, 2.55 mmol) and **3** (1.0 g, 4.64 mmol) were combined as above, to yield 828 mg of **A2A**·2HCl (66%), which was pure by ^{1}H and ^{13}C NMR analysis. Recrystallization from 10% MeOH–H_2O afforded 667 mg (53%) of the desired product as off-white crystals. ^{1}H NMR (D_2O): δ 1.61 (s, br, 8H), 2.13 (s, br, 4H), 2.41 (s, br, 4H), 3.89 (s, br, 4H), 7.07 (d, J = 8.4 Hz, 2H), 7.14 (t, J = 7.7 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.53 (t, J = 7.7 Hz, 2H); ^{13}C NMR (D_2O): δ 22.23, 23.34, 26.53, 29.94, 49.25, 114.39, 117.02, 120.61, 124.76, 127.51, 135.06, 138.36, 153.18, 158.27 ppm; MS (CI^+): Calc. for $C_{28}H_{30}N_4$: 422. Found: 423 (M + 1); mp: 274 °C (decomp.); HPLC: 18.8 min, 96.0 area%. Elemental Analysis: Calc. for $C_{28}H_{32}N_4Cl_2·4.1H_2O$: C, 59.07; H, 7.12; N, 9.84. Found: C, 58.69; H, 7.14; N, 9.71.

Propylene-linked bis-THA bis-hydrochloride (A3A·2HCl). 1,3-Diaminopropane (209 μL, 2.51 mmol) and **3** (1.1 g, 5.07 mmol) were combined as above, to yield 821 mg of **A3A**·2HCl (65%), which was pure by ^{1}H and ^{13}C NMR analysis. Recrystallization from 10% MeOH–H_2O afforded 657 mg (52%) of the desired product as off-white crystals. ^{1}H NMR (CD_3OD): δ 1.7–1.9 (m, 8H), 2.29 (quintet, J = 6.0 Hz, 2H), 2.4–2.5 (m, 4H), 2.75–2.85 (m, 4H), 4.02 (t, J = 6.0 Hz, 3H), 7.47 (ddd, J = 1.5, 6.9, 8.6 Hz, 2H), 7.72 (dd, J = 1.2, 8.5 Hz, 2H), 7.80 (ddd, J = 1.29, 7.0, 8.3 Hz, 2H), 8.16 (apparent d, J = 8.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 22.81, 23.76, 25.83, 30.37, 33.16, 46.98, 114.34, 117.69, 121.64, 127.95, 127.71, 135.06, 140.97, 152.90, 159.02; MS (CI^+): Calc. for $C_{29}H_{32}N_4$: 436. Found: 437 (M + 1). mp: 269 °C (decomp.). HPLC: 19.3 min, 99.9 area%. Elemental Analysis: Calc. for $C_{29}H_{34}N_4Cl_2·2.5H_2O$: C, 62.81; H, 7.09; N, 10.10; Found: C, 62.87; H, 7.53; N, 9.88.

Butylene-linked bis-THA bis-hydrochloride (A4A·2HCl). 1,4-Diaminobutane (213 μL, 2.12 mmol) and **3** (1.0 g, 4.67 mmol) were combined as above, to yield 723 mg of **A4A**·2HCl (65%), which was pure by ^{1}H and ^{13}C NMR analysis. Recrystallization from 10% MeOH–H_2O afforded 586 mg (53%) of the desired product as off-white crystals. ^{1}H NMR (D_2O): δ 1.63 (s, br, 12H), 2.03 (s, br, 4H), 2.48 (s, br, 4H), 3.65 (s, br, 4H), 7.14–7.20 (m, 4H), 7.56 (d, J = 7.8, 2 H), 7.62 (t, J = 8.8, 2H); ^{13}C NMR (CD_3OD): δ 22.74, 23.86, 25.97, 29.13, 30.48, 49.07, 114.03, 118.08, 121.49, 127.04, 127.19, 134.70, 140.86, 153.00, 158.19; MS (CI^+): Calc. for $C_{30}H_{34}N_4$: 450. Found: 451 (M + 1). mp: 271 °C (decomp.). HPLC: 20.9 min, 99.8 area% Elemental Analysis: Calc. for $C_{30}H_{36}N_4Cl_2·0.5\ H_2O$: C, 67.66; H, 7.00; N, 10.52. Found: C, 67.78; H,6.89; N, 10.60.

Pentylene-linked bis-THA bis-hydrochloride (A5A·2HCl). 1,5-Diaminopentane (271 μL, 2.31 mmol) and **3** (1.0 g, 4.62 mmol) were combined as above, to yield 834 mg of **A5A**·2HCl (78%), which was pure by ^{1}H and ^{13}C NMR analysis. Recrystallization from 10% MeOH–H_2O afforded 677 mg (54.6%) of the desired product as off-white crystals. ^{1}H NMR (D_2O): δ 1.41–1.57 (m, 2H), 1.61–1.81 (m, 12H), 2.28 (s, br, 4H), 2.61 (s, br, 4H), 3.63–3.73 (m, 4H), 7.18 (t, J = 7.8 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H), 7.59 (t, J = 7.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H); ^{13}C NMR (CD_3OD): δ 23.12, 23.94, 25.46, 25.98, 31.88, 31.96, 49.58, 115.05, 119.37, 124.11, 125.91, 126.07, 132.058, 142.81, 155.48, 156.17; MS (CI^+): Calc. for $C_{31}H_{36}N_4$: 464. Found: 465 (M + 1). mp: 241 °C (decomp). HPLC: 22.4 min, 98.7 area%. Elemental Analysis: Calc. for $C_{31}H_{38}N_4Cl_2·2.1\ H_2O$: C, 64.71; H, 7.39; N, 9.74. Found: C, 64.38; H, 6.99; N, 9.90.

Hexylene-linked bis-THA bis-hydrochloride (A6A·2HCl). 1,6 Diaminohexane (2.68 g, 23.0 mmol) and **3** (10.0 g, 46.08 mmol) were combined as above, to yield 11.06 g (87%) **A6A**·2HCl, which was pure by ^{1}H and ^{13}C NMR analysis. Recrystallization from 10% MeOH–H_2O afforded 8.94 g (71%) of the desired product as off-white crystals. ^{1}H NMR (D_2O): δ 1.41 (s, br, 4H), 1.71 (m, 12H), 2.23 (s, br, 4H), 2.61 (s,br, 4H), 3.58 (t, J = 6.7 Hz, 4H), 7.21 (m, 4H), 7.55 (apparent t, J = 7.7 Hz, 2Hz), 7.74 (apparent d, J = 8.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 22.61, 23.76, 25.73, 28.19, 30.09, 32.21, 113.62, 117.80, 120.88, 127.12, 127.27, 134.86, 140.50, 152.45, 158.71; MS (CI^+): Calc. for $C_{32}H_{38}N_4$: 478. Found: 479 (M + 1). mp: 153.3–154.6 °C. HPLC: 23.6 min, 99.5 area%. Elemental Analysis: Calc. for $C_{32}H_{40}N_4Cl_2·2H_2O$: C, 65.41; H, 7.55; N, 9.53. Found: C, 65.76; H, 7.51; N, 9.48.

Octylene-linked bis-THA bis-hydrochloride (A8A·2HCl). Using the previously described THA alkylation protocol,[3] THA (1.0 g, 3.96 mmol) and powdered KOH (1.55 g, 27.7 mmol) were dissolved in 20 mL of DMSO, and placed under nitrogen. This mixture was heated periodically with a heat gun for 30 min, avoiding

boiling. After cooling to room temperature, 1,8-dibromooctane (364 μL, 1.98 mmol) was added by syringe. The homogeneous brown solution was stirred for 30 h at room temperature, quenched by pouring into 100 mL of H_2O, and extracted with ethyl acetate (4×50 mL). The combined extracts were washed with H_2O (2×30 mL), dried ($MgSO_4$), filtered and the solvent removed in vacuo to give a brown liquid; purification by flash chromatography (8% MeOH–CH_2Cl_2 with 7 mL conc. NH_3 per L) gave 805 mg of **A8A** (80%), which was pure by ^1H and ^{13}C NMR analysis. The bis-HCl salt was prepared in methanol by treatment with HCl gas. Recrystallization from 10% MeOH–H_2O afforded 598 mg (52%) of the desired product **A8A**·2HCl as yellow crystals. ^1H NMR (D_2O): δ 1.33 (s, br, 8H), 1.64 (s, br, 4H), 1.78 (s, br, 8H), 2.27 (s, br, 4H), 2.68 (s, br, 8H), 3.53 (t, J = 7.1 Hz, 4H), 7.18 (apparent t, J = 7.9 Hz, 2H), 7.24 (apparent d, J = 8.4 Hz, 2H), 7.52 (apparent t, J = 7.7 Hz, 2H), 7.71 (apparent d, J = 8.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 22.61, 23.70, 25.56, 28.13, 30.16, 30.60, 32.08, 113.64, 117.53, 127.19, 135.06, 140.40, 152.54, 158.43; MS (CI^+): Calc. for $C_{38}H_{42}N_4$: 506, Found: 507 (M + 1); mp: 148.7–150.9 °C; HPLC: 25.9 min., 98.4 area%; Elemental Analysis: Calc. for $C_{34}H_{44}N_4Cl_2·2.1H_2O$: C, 66.13; H, 7.87; N, 9.07. Found: C, 65.84; H, 7.56; N, 9.39.

Nonylene-linked bis-THA bis-hydrochloride (A9A·2HCl). Use of 1,9-dibromononane in the **A8A** procedure described above provided 585 mg of **A9A**·2HCl. Recrystallization from 10% MeOH–H_2O afforded 414 mg (35%) of the desired product as yellow crystals. ^1H NMR (D_2O): δ 1.30–1.35 (m, 10H), 1.63–1.65 (m, 4H), 1.81 (s, br, 8H), 2.29 (s, br, 4H), 2.73 (s, br, 4H), 3.52 (t, J = 7.0 Hz, 4H), 7.20 (apparent t, J = 7.8 Hz, 2H), 7.23 (apparent d, J = 8.4 Hz, 2H), 7.55 (apparent t, J = 7.7 Hz, 2H), 7.71 (apparent d, J = 8.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 22.40, 23.50, 25.39, 28.06, 29.93, 30.50, 30.77, 31.96, 113.41, 117.33, 120.81, 126.99, 134.82, 140.20, 152.26, 158.26; MS ($CI^-(NH_3)$): Calc. for $C_{35}H_{44}N_4$: 520. Found: 519 (M−1); HPLC: 26.5 min, 96.1 area%; Elemental Analysis: Calc. for $C_{35}H_{46}N_4Cl_2·2H_2O$: C, 66.76; H, 8.00; N, 8.90. Found: C, 66.77; H, 7.62; N, 9.30.

Decylene-linked bis-THA bis-hydrochloride (A10A·2HCl). 1,10-Diaminodecane (397 mg, 2.30 mmol) and **3** (1.0 g, 4.61 mmol) were combined as above, to yield 909 mg of **A10A**·2HCl (65%), which was pure by ^1H and ^{13}C NMR analysis. Recrystallization from 10% MeOH–H_2O afforded 416 mg (30%) of the desired product as off-white crystals ^1H NMR (CD_3OD): δ 1.31–1.40 (m, 12H), 1.81 (quintet, J = 7.3 Hz, 4H), 1.90–1.99 (m, 8H), 2.68 (t, J = 5.7 Hz, 4H), 3.01 (t, J = 5.4 Hz, 4H), 3.93 (t, J = 7.4 Hz, 4H), 7.57 (ddd, J = 1.6, 6.9, 8.6 Hz, 2H), 7.76 (dd, J = 1.3, 8.6 Hz, 2H), 7.83 (ddd, J = 1.2, 6.9, 8.3 Hz, 2H), 8.37 (apparent d, J = 8.7 Hz, 2H); ^{13}C NMR (CD_3OD): δ 22.41, 23.54, 25.44, 28.76, 29.88, 30.84, 31.06, 32.10, 49.71, 113.40, 117.62, 120.69, 126.85, 127.04, 134.63, 140.38, 152.26, 158.54; mp: 128.6–130.0 °C; MS(CI^+): Calc. for $C_{36}H_{46}N_4$: 534. Found: 535 (M + 1); HPLC: 27.8 min, 96.2 area%; Elemental Analysis: Calc. for $C_{36}H_{48}N_4Cl_2·2.3H_2O$: C, 66.61; H, 8.17; N, 8.63. Found: C, 66.66; H, 8.15; N, 8.56.

Enzyme preparation and AChE/BChE inhibition studies. AChE and BChE enzyme preparations were prepared from cortex and serum respectively of decapitated rats. Frontal cortex (brain dissected on ice) was homogenized in sodium phosphate buffer (39 vol. 75 mM, pH 7.4). Rat serum was obtained by centrifugation of blood (3500×g, 10 min.). The cholinesterase assays were performed using the colorimetric method of Ellman,[13] with minor modification. For determination of AChE inhibition, cortex homogenate was preincubated for 5 min with ethopropazine (0.1 mM), a selective inhibitor of BChE. Similarly, for determination of BChE inhibition, serum was preincubated with BW284c51 (0.01 mM), a selective inhibitor of AChE. A mixture of 4 mL containing acetylthiocholine iodide (0.3 mM) or butrylthiocholine iodide (0.4 mM), 1 mL sodium phosphate buffer (0.1 mM, pH 7.4), a solution of the compound being tested (0.1 mL), and homogenate or serum (0.1 mL) was incubated at 37 °C for 8 min. The reaction was terminated by the addition of sodium dodecyl sulfate (3% w/v, 1 mL), after which the 5.5′-dithio-bis(2-nitrobenzoic acid) indicator (0.2% w/v, 1 mL) was added. Enzyme activity was determined by measuring the absorbance at 420 nm after 10 min, relative to the drug-free control. Triplicate measurements were performed at typically a total of 8 drug concentrations; IC_{50} values were determined from a plot of Enzyme Activity versus −log[drug]. The AChE K_i values of **A7A**·2HCl and THA·HCl, and modes of inhibition were determined using Lineweaver–Burke plots.

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