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Synthesis and structure-activity relationship of 2,6-disubstituted pyridine derivatives as inhibitors of β-amyloid-42 aggregation

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ABSTRACT

It is assumed that amyloid-β aggregation is a crucial event in the pathogenesis of Alzheimer's disease. Novel 2,6-disubstituted pyridine derivatives were designed to interact with the β-sheet conformation of Aβ via donor-acceptor-donor hydrogen bond formation. A series of pyridine derivatives were synthesized and tested regarding their potential to inhibit the aggregation of Aβ. The 2,6-diaminopyridine moiety was identified as a key component to inhibit Aβ aggregation. Overall, compounds having three 2,6-disubstituted pyridine units separated by at least one C2- or C3-linker displayed the most potent inhibition of Aβ aggregation.

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder affecting an estimated 36 million patients in 2010.[1] Current treatment options are limited to drugs treating the symptoms and do not slow or reverse disease progression. Thus, a disease modifying drug would be a major therapeutic breakthrough towards treatment of the disease. Although the mechanisms of neurodegeneration in AD are not yet fully discovered, some major pathological signs, such as senile plaques (SP) composed of amyloid-β (Aβ) peptides and neurofibrillary tangles composed of hyperphosphorylated tau protein, are characterized.[2,3] SPs consist predominantly of the 40 to 42 amino acid Aβ peptides, which are derived from the amyloid precursor protein (APP). The formation of Aβ fibrils, via aggregation of soluble Aβ in an antiparallel β-sheet conformation[4], and their deposition into neurotoxic amyloid plaques is considered an initial event in the progression of AD.[5] Recent studies on Aβ toxicity suggest that an early stage involves the aggregation of extracellular soluble Aβ peptide into low molecular weight soluble oligomers or high molecular weight prefibrillar intermediates.[6-8] A central therapeutic aim in AD is the removal of toxic β-amyloid deposits[9], which can be achieved by secretase inhibitors (inhibition of Aβ production), drugs promoting β-amyloid clearance via active or passive immunotherapy or inhibition of Aβ aggregation and toxicity. Preventing Aβ aggregation is therapeutically attractive because the process is believed to be a purely pathological event and does not interfere with the physiological role of APP.[10]

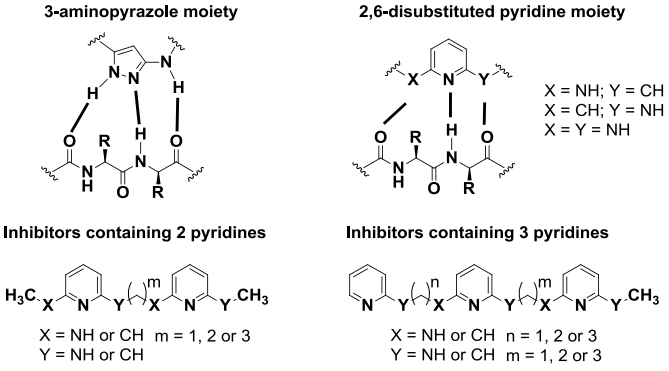

Figure 1. Possible hydrogen bond interactions of 3-aminopyrazole or 2,6-disubstituted pyridine derivatives with the β-sheet conformation of Aβ and inhibitor design.

We had already prepared small molecule inhibitors of Aβ aggregation based on the connection of two substituted 3-aminopyrazole moieties via a linker to enable donor-acceptor-donor hydrogen bond interactions complementary to that of the β-sheet conformation of Aβ and additional π-π

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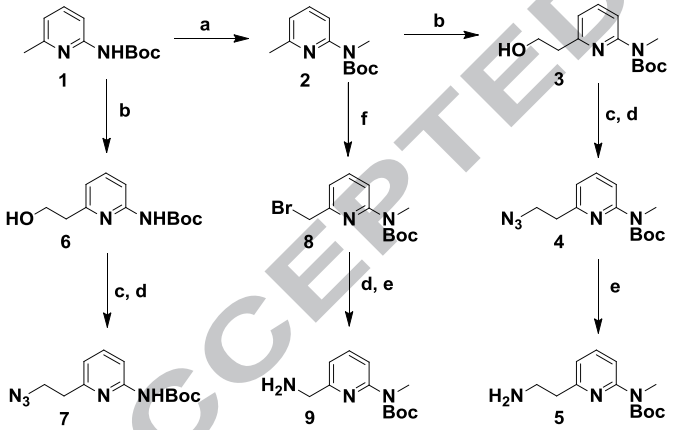
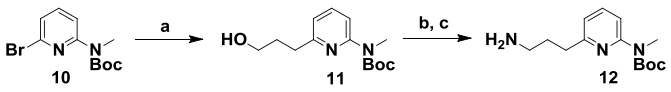
stacking/hydrophobic interactions with amino acid residues of Aβ.[11] This previous series of Aβ aggregation inhibitors based on the 3-aminopyrazole moiety, however, displayed low solubility at physiological pH (< 10 μM) and low metabolic stability leading to poor bioavailability. The aim of the novel 2,6-disubstituted pyridine series was to maintain potency but to increase solubility as well as metabolic stability. Overall, the novel inhibitors should contain up to 3 hydrogen bond donors and 3 hydrogen bond acceptors. The individual 2,6-disubstituted pyridine moieties should be connected via C1- to C3-linker units to identify the optimal linker length.

To synthesize the novel Aβ aggregation inhibitors containing two or three 2,6-disubstituted pyridine moieties, it was necessary to prepare suitable building blocks. The synthesis of monomeric 2,6-disubstituted pyridine building blocks is shown in Schemes 1 and 2. The starting material, compound **1**, to synthesize compounds **2** to **9** was prepared as described.[12] Building blocks **5**, **7** and **9** were prepared via 3-5 steps. First, compound **1** was alkylated with methyliodide using sodium hydride.[12] Next compound **2** was treated with lithiumdiisopropylamine (LDA) at –78°C, followed by the addition of dimethylformamide to obtain the C1-elongated aldehyde intermediate. Reduction with sodium tetrahydroborohydride at –78°C in the presence of acetic acid and methanol yielded the corresponding alcohol **3**.[13]Activation of the alcohol with methanesulfonylchloride followed by displacement with sodium azide under neutral conditions yielded **4**.[14] Reduction of the azide under Staudinger conditions afforded the amine building block **5** containing a C2-linker.[14] Compound **6** was prepared from **1** using the same conditions employed for the synthesis of **3**. The azide building block **7** containing a C2-linker was prepared in the same manner as described for **4**. NBS-bromination of **2** as described[15] afforded **8**, which was converted to the C1-linker amine building block **9** as described for **5**.



Scheme 1. Reagents and conditions: (a) NaH, DMF, CH_3I, 0 °C to rt, 16 h, 74%; (b) (i) LDA, THF, -78 °C; (ii) DMF, -78 °C; (iii) HOAc, MeOH, $NaBH_4$, -78 °C to rt, 35-44%; (c) CH_3SO_2Cl, TEA, DCM, 0 °C to rt, 1 h; 65-90% (d) NaN_3, DMA, 75 °C, 16 h, 89-95%; (e) TPP, THF, H_2O, rt, 24 h, 82-90%; (f) NBS, AIBN, CCl_4, 100 °C, 5 h, 29%.

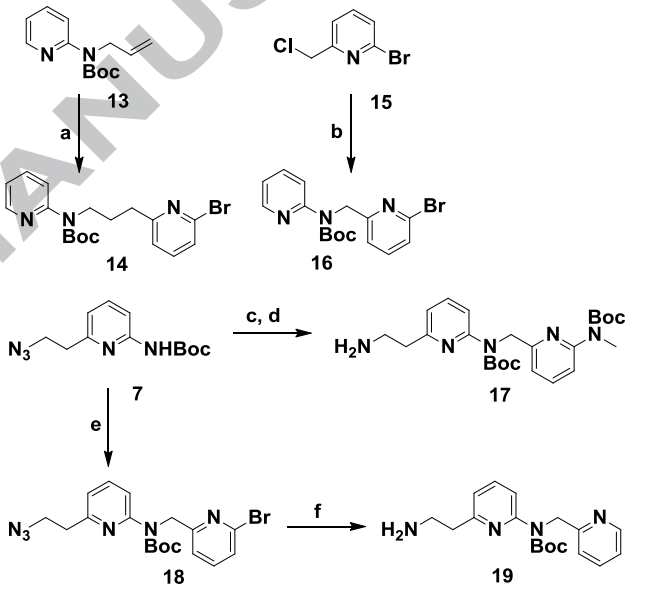


Scheme 2. Reagents and conditions: (a) (i) allylalcohol, 9-BBN, THF 0 °C, 4 h; (ii) $Pd[P(Ph)_3]_4$, THF, NaOH, DMA, 95 °C, 90 min, 79%; (b) phthalimide, TPP, THF, DEAD, rt, 16 h, 86%; (c) N_2H_4 x H_2O, MeOH, rt, 16 h, 60%.

Building block **12** was prepared via 3 steps (Scheme 2). Compound **10** was prepared as described.[16] The C3-linker was introduced via Suzuki coupling using tetrakis(triphenylphosphine)palladium[0] ($Pd[P(Ph)_3]_4$) utilizing an intermediate formed by the reaction of allylalcohol with 9-borabicyclo[3.3.1]-nonane (9-BBN) to afford the corresponding alcohol **11**.[17]

Mitsunobu reaction of **11** using diethyl azodicarboxylate (DEAD), triphenylphosphine (TPP) and phthalimide followed by the cleavage of the protecting group with hydrazine hydrate afforded the C3-linker amine building block **12**.[18]

The preparation of the required dimeric building blocks **14**, **16**, **17** and **19** via 1-2 steps synthesis is shown in Scheme 3. Starting material **13** was prepared as described[19] and treated with 9-BBN to form a boron intermediate. This intermediate was then reacted with commercially available 2,6-dibromopyridine as described for **11** to afford the dimeric building block **14** containing a C3-linker. The corresponding building block **16** containing a C1-linker was prepared from **15** using commercially available Boc-2-amino-pyridine and the alkylation conditions employed for the preparation of **2**. Starting material **15** was synthesized as described.[20] Attempts to use the methanesulfonate derivative of **3** under the basic conditions described for the preparation of **16** resulted in the formation of the corresponding vinylpyridine derivative of **3**.

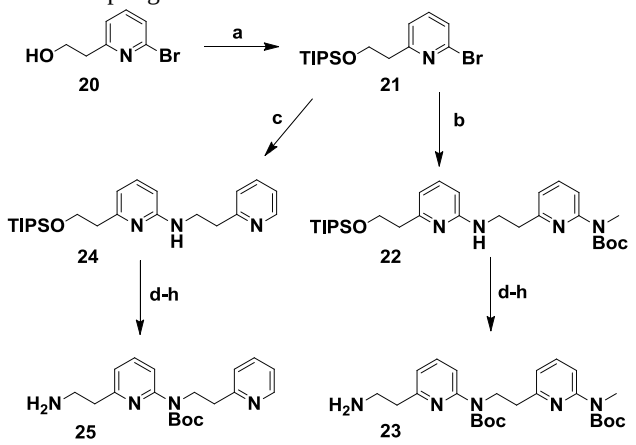


Scheme 3. Reagents and conditions: (a) (i) 9-BBN, THF 0 °C, 4 h; (ii) THF, NaOH, $Pd[P(Ph)_3]_4$, DMA, 2,6-dibromopyridine, 95 °C, 90 min, 69%; (b) NaH, DMF, Boc-2-amino-pyridine, 65 °C, 3 h, 82%; (c) NaH, DMF, **8**, 60 °C, 2 h, 72%; (d) TPP, THF, H_2O, rt, 24 h, 68%; (e) NaH, DMF, **15**, 60 °C, 2 h, 82%; (f) H_2, Pd/C, EtOH, TEA, rt, 90%.

The dimeric building blocks **17** and **19** (Scheme 3) were both prepared from compound **7**. Alkylation of **7** with **8** followed by Staudinger reduction as described for **5** afforded the dimeric building block **17** containing a C1- and a C2-linker. Alkylation of compound **7** with **15** afforded compound **18**. Removal of the bromo-substituent and reduction of the azide was accomplished by catalytic hydrogenation with palladium on carbon to afford the dimeric building block **19** containing a C1- and a C2-linker.

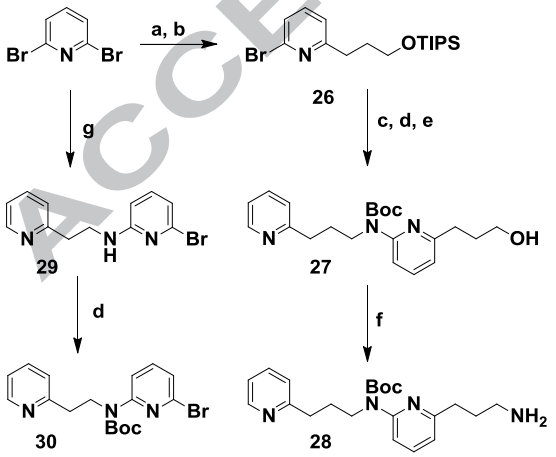
The starting material **20** for the preparation of dimeric building blocks **23** and **25** (Scheme 4) was prepared as described.[15] Protection of the alcohol with the triisopropylsilyl (TIPS) moiety gave **21**. Compound **21** was then reacted with **5** using tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$), 2,2-Bis-(diphenylphosphino)-1,1-napthalene (BINAP) and sodium tert.-butoxide under Buchwald conditions.[21] The use of 10 mol% palladium catalyst and a short reaction time (45 min) were critical for good conversion and yield. Extended reaction times lead to significant decomposition of **22** in the presence of the strong base sodium tert.-butoxide. No formation of dimeric

coupling products was observed under the reaction conditions. The free NH-group in **22** was Boc-protected at elevated temperature. The best results were obtained by dissolving **22** and di-tert-butyl dicarbonate (Boc_2O) in dichloromethane and evaporation of the solvent followed by heating of the oily reaction mixture. After cleavage of the TIPS-group with tetra-butylammoniumfluoride, the free OH-group was converted via 3 additional steps to the amine as described for **5** to afford the dimeric building block **23** containing two C2-linkers. The synthesis of the dimeric building block **25** containing two C2-linkers was performed as described for **23** except that commercially available 2(2-aminoethyl)-pyridine was used for the Pd-coupling reaction.



Scheme 4. Reagents and conditions: (a) TIPS-Cl, imidazole, DMF, rt, 16 h, 87%; (b) $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, **5**, 85 °C, 45 min, 78%; (c) $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, 2-(2-aminoethyl)pyridine, 85 °C, 45 min, 81%; (d) Boc_2O, 70 °C, 16 h, 93%; (e) TBAF, THF, CH_3CN, rt, 16 h, 88-92%; (f) CH_3SO_2Cl, TEA, DCM, 0 °C to rt,1 h, 85-90%; (g) NaN_3, DMA, 75 °C, 16 h, 65-92%; (h) TPP, THF, H_2O, rt, 24 h, 85-93%.

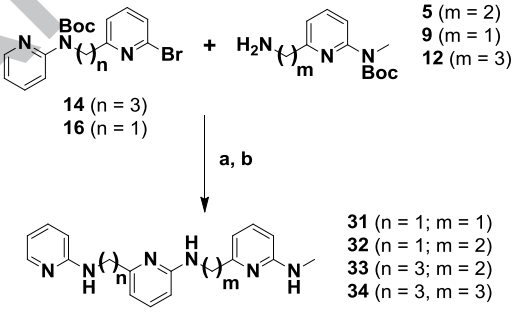
The preparation of the dimeric building blocks **28** and **30** via 2-6 steps is shown in Scheme 5. Commercially available 2,6-dibromopyridine was converted to **26** via Suzuki coupling with the reaction product of 9-BBN and allylalcohol followed by TIPS-protection of the coupling product as described for **11**. Compound **26** was then coupled under Buchwald conditions with 3-(pyridin-2-yl)propylamine, which was prepared as described.[18]



Scheme 5. Reagents and conditions: (a) (i) allylalcohol, 9-BBN, THF 0 °C, 4 h; (ii) $Pd[P(Ph)_3]_4$, THF, NaOH, DMA, 95 °C, 90 min, 49%; (b) TIPS-Cl, imidazole, DMF, rt, 16 h, 79%; (c) $Pd_2(dba)_3$, BINAP, NaOtBu, 3-(pyridin-2-yl)propylamine, toluene, 85 °C, 45 min, 83%;(d) Boc_2O, 70 °C, 16 h, 53-92%; (e) TBAF, THF,CH_3CN, rt, 16 h, 94%; (f) (i) TPP, phthalimide, THF, DEAD, rt, 16 h; (ii) N_2H_4 x H_2O, MeOH, rt, 16 h, 62%; (g) $KHCO_3$, DMA, 2-(2-aminoethyl)pyridine, 110 °C, 5 h, 21%.

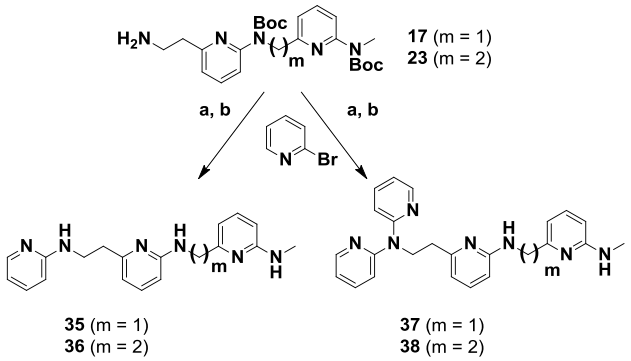
Subsequent Boc-protection and cleavage of the TIPS-group as described for **23** afforded **27**. Conversion of the OH-group to the corresponding amine was performed as described for **12** to obtain the dimeric building block **28** containing two C3-linkers. Treatment of 2,6-dibromopyridine with 2(2-aminoethyl)pyridine at elevated temperature in the presence of potassium hydrogencarbonate afforded **29**, which was Boc-protected as described for **23** to obtain the corresponding dimeric building block **30** containing a C2-linker.

The synthesis of Aβ aggregation inhibitors containing either two or three 2,6-disubstitued pyridines and different linkers is shown in Schemes **6**, **7** and **8**. The inhibitors **31-34** (Scheme 6) were prepared by using building blocks **5**, **9**, **12**, **14**, and **16**. First, the building blocks were combined via Buchwald coupling[21] at 85 °C for 45 minutes to yield the corresponding Boc-protected inhibitors. Under the reaction conditions no di-substituted coupling products were observed. Cleavage of the Boc-group under acidic conditions afforded the desired inhibitors as hygroscopic HCl-salts. The best way to obtain the inhibitors was to remove the organic solvents by syringe and to dissolve the precipitated HCl-salt in water. Lyophilization of the aqueous solution then afforded the Aβ aggregation inhibitors as pale yellow/orange powders. Thus, **31** was prepared from **9** and **16**, **32** was prepared from **5** and **16**, **33** was prepared from **5** and **14**, and **34** was prepared from **12** and **14** (Scheme 6).



Scheme 6. Reagents and conditions: (a) $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, 85 °C, 45 min, 50-82%; (b) 2 M HCl, Et_2O, $CHCl_3$, rt, 16 h, 69-85%.

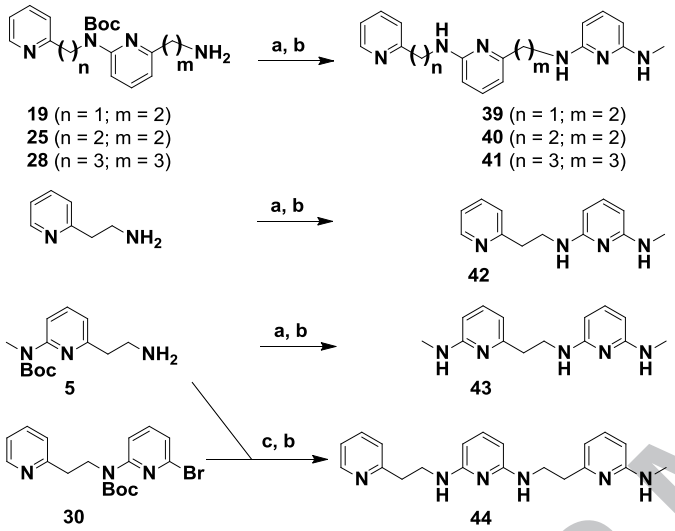
The inhibitors **35-38** were prepared from **17** and **23** via coupling with 2-bromopyridine under Buchwald conditions (Scheme 7). Congruent to literature data[21], the use of 2-bromopyridine yielded a mixture (~1:1) of mono- and di-substituted coupling products, which could be separated by preparative thin layer chromatography. The faster moving band was always the di-substituted coupling product, *i.e.* Boc-protected **37** or **38**. Thus, the Boc-protected derivatives of **35** and **37** were obtained from **17** and 2-bromopyridine, whereas the Boc-protected derivatives of **36** and **38** were obtained from **23** and 2-bromopyridine (Scheme 7).



Scheme 7. Reagents and conditions: (a) $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, 85 °C, 45 min, 7-22%; (b) 2 M HCl, Et_2O, $CHCl_3$, rt, 16 h, 57-97%.

After acidic cleavage of the Boc-protection groups, the desired inhibitors **35-38** were obtained as hygroscopic solids.

The Boc-protected inhibitors **39-41** were prepared via palladium catalyzed coupling of **19**, **25** and **28** with **10** at 110 °C for 45 minutes (Scheme 8). Since the coupling resulted in the formation of a 2,6-diaminopyridine moiety, a higher reaction temperature was required.[22] The Boc-protected dimeric inhibitors **42** and **43** were prepared in the same manner by coupling 2-(2-aminoethyl)pyridine and **5** with **10**. The Boc-protected inhibitor **44** was prepared by coupling of **30** with **5**. Unlike the reactions with 2-bromopyridine, no di-substituted palladium coupling products were observed. The synthesis of inhibitors **39-44** was completed by cleavage of the Boc-protection group with acid (Scheme 8).



Scheme 8. Reagents and conditions: (a) $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, **10**, 110 °C, 45 min, 22-89%; (b) 2 M HCl, Et_2O, $CHCl_3$, rt, 16 h, 72-84%.(c) $Pd_2(dba)_3$, BINAP, NaOtBu, toluene, **5**, 110 °C, 45 min, 94%

The inhibition of Aβ aggregation in the presence of **31-44** was determined using a thioflavin T (ThT) fluorescence assay and crude $A\beta_{1-42}$ peptide film at 33 μM as described.[11] First, **31-38** were screened and their capability to reduce the ThT fluorescence signal of the control reaction ($A\beta_{1-42}$ aggregation in DMSO) at 330 μM was determined (Figure 2). The data in Figure 2 showed that **31** containing two C1-linkers, **32** having a C1-linker to the left and C2-linker to the right of the central pyridine, and **35** having a C2-linker to the left and a C1-linker to the right of the central pyridine displayed rather low inhibition of $A\beta_{1-42}$ aggregation properties (>60% of the control value). Thus, compounds with one or two short C1-linkers most likely could not form strong donor-acceptor-donor interactions with the β-sheet conformation of $A\beta_{1-42}$. In contrast, **37** containing a bis-pyridyl moiety, **33** having a C3-linker to the left and a C2-linker to the right, and **34** containing two C3-linkers displayed better inhibition of $A\beta_{1-42}$ aggregation properties (23-29% of the control value). This indicated a preference for compounds with either longer and more flexible linkers (**33**, **34**) or the capability to form additional π-π interactions (**37**). For all compounds containing three 2-aminopyridine moieties separated by different linkers, **36** and **38** displayed the best inhibition of $A\beta_{1-42}$ aggregation properties (16% of the control value). In contrast to **35**, compound **36** containing two C2-linkers displayed a similar potency as **38** containing a bis-pyridyl moiety. This suggests that proper hydrogen bond donor interactions of each of the three 2-aminopyridyl moieties in **36** have a similar effect than additional π-π interactions for **37** and **38**. The distance between donor and acceptor should be ideally in the range of 3.5-4.0 Å and the distance between acceptor and donor should be 2.6-2.9 Å.[23] Superiority of the C2-linker in this series was also evident from the stronger inhibition of $A\beta_{1-42}$ aggregation of **38** compared to **37**.

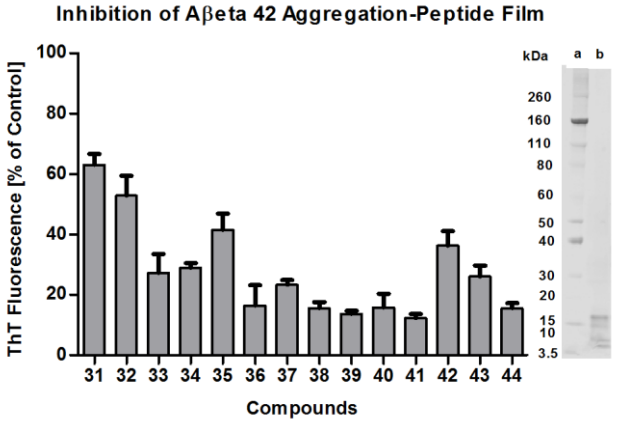


Figure 2. *In vitro* screening assays using $A\beta_{1-42}$ peptide film. The concentration of $A\beta_{1-42}$ peptide film was 33 μM. The test concentration for compounds **31-44** was 330 μM and the incubation time was 24 h. Data are expressed as percentage (mean ± S.D.) of control conditions: $A\beta_{1-42}$ aggregation with DMSO only. Freshly prepared $A\beta_{1-42}$ peptide film (4 μg) was analyzed by SDS-PAGE to confirm the presence of oligomeric $A\beta_{1-42}$ present (*a*, molecular weight marker; *b*, $A\beta_{1-42}$ peptide film).

In order to evaluate the effect of different distribution patterns of the three hydrogen bond donors and acceptors, compounds **39-44** were prepared and their inhibition of $A\beta_{1-42}$ aggregation properties tested (Figure 2). Taken the results for **31-38** into account, the C2-linker was preferentially incorporated into compounds **39-44**. In contrast to **31-38**, each of compounds **39-44** contained a 2,6-diaminopyridine moiety. The 2,6-diaminopyridine moiety in **39-43** is located at a terminal right position, whereas in **44** the position is central. To get an idea on how many hydrogen donors and acceptors are required for efficient inhibition of $A\beta_{1-42}$ aggregation, the truncated compounds **42** and **43** were prepared as well.

The results for **39-41** and **44** clearly showed that all compounds containing the 2,6-diaminopyridine unit displayed efficient inhibition of $A\beta_{1-42}$ aggregation (12-16% of the control value). The position of the 2,6-diaminopyridine moiety, *i.e.* terminal right (**39-41**) or central (**44**), had no significant impact on their inhibition of $A\beta_{1-42}$ aggregation properties in the screening assay. The same applied for the use of C2-linkers (**40**) or C3-linkers (**41**). Interestingly, compound **39** was equally potent (14% of the control value) when compared to **40** and **41**. This was in sharp contrast to compound **32** (53% of the control value), although the linker composition and number of hydrogen bond donors and acceptors were identical. This suggests the 2,6-diaminopyridine moiety is a preferred unit to enable donor-acceptor interactions with the β-sheet conformation of $A\beta_{1-42}$. Further evidence came from the ''truncated'' inhibitors **42** and **43** containing a 2,6-diaminopyridine unit. Compound **42** displayed a higher inhibition of $A\beta_{1-42}$ aggregation (36% of the control value) with just 2 hydrogen bond donors and acceptors when compared to **31**, **32** and **35** having 3 hydrogen bond donors and acceptors (Figure 2). Compound **43** having 3 hydrogen bond donors and 2 hydrogen bond acceptors was equally potent as **33** and **34**, indicating the importance of hydrogen bond donors for inhibition of $A\beta_{1-42}$ aggregation.

In order to better compare inhibitors displaying >80% inhibition of the control value in the ThT-screening assay, the IC_{50} for **36**, **38**, **39**, **40**, **41** and **44** was determined using the ThT fluorescence assay. To compare our inhibitors with Aβ aggregation inhibitors from literature, the IC_{50} of Congo Red and Curcumin were determined as well (Figure 3).

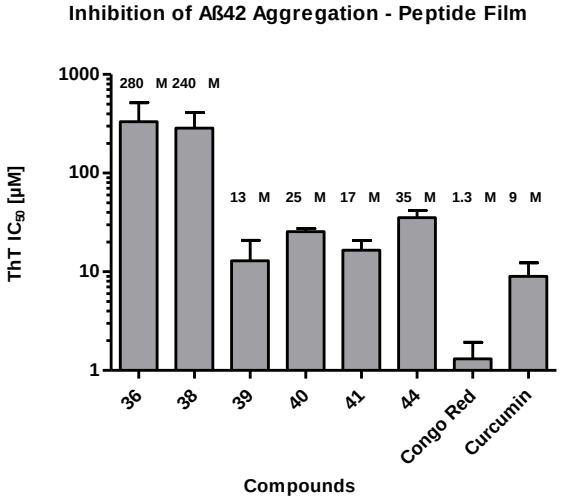


Figure 3. IC_{50} determination assay using $A\beta_{1-42}$ peptide film. The concentration of $A\beta_{1-42}$ peptide film was 33 µM. The test concentration for compounds **36**, **38**, **39**, **40**, **41**, **44**, Congo Red and Curcumin were 330 µM, 82.5 µM, 20.63 µM, 5.16 µM, 1.29 µM, 0.32 µM and 0.08 µM with an incubation time of 24 h. The IC_{50} values were determined from the fluorescence values obtained. The ThT IC_{50}-data (µM) are expressed as mean ± standard deviation and indicated on top of each graph.

The IC_{50} determination for **36**, **38**, **39**, **40**, **41** and **44** clearly showed that compounds containing a 2,6-diaminopyridine moiety (**39-44**) displayed a better inhibition of $A\beta_{1-42}$ aggregation (13-35 µM) when compared to **36** and **38** (240-280 µM) having a 2-aminopyridine moiety (Figure 3). Compound **44** bearing a central 2,6-diaminopyridine was slightly less active (35 µM) than **39**, **40** and **41** containing a terminal right 2,6-diaminopyridine moiety. Compounds **39-41** displayed comparable $A\beta_{1-42}$ aggregation properties with IC_{50} values of 13 µM, 25 µM, and 17 µM, respectively. The control compound Curcumin inhibited $A\beta_{1-42}$ aggregation in the same range (9 µM). Congo Red was the most potent compound (1.3 µM) in this assay.

The aqueous solubility of selected compounds **31**, **40**, **41**, **43** and **44** at physiological pH (PBS, pH 7.4) were determined as 18 µM, >200 µM, 103 µM, 195 µM and > 200 µM, respectively. Compared to the previous 3-aminopyrazole class of $A\beta_{1-42}$ aggregation inhibitors[11], the introduction of 2,6-disubstituted pyridines moieties allowed to improve the aqueous solubility of $A\beta_{1-42}$ aggregation inhibitors from <10 µM (3-aminopyrazoles) to >200 µM (**40** and **44**) while maintaining similar potency.

Overall, compound **40** appeared to be a good compromise between inhibition of $A\beta_{1-42}$ aggregation and high aqueous solubility and was further profiled *in vitro*. Though the metabolic stability of **40** in human liver S9 fraction was low ($t_{1/2}$ = 10 min; 0.07 mL/min/mg), **40** displayed very high permeability (P_{app} A to B = 49.2 x 10^{-6} cm/sec), was not a P-gp substrate (Efflux ratio = 0.78) and displayed a favorable LogD (2.96). Thus, the pharmacokinetic properties of compound **40** were tested in male Swiss mice (Figure 4, Table 1).[24] After intravenous application at 2 mg/kg, compound **40** rapidly entered the brain (1845 ng/g after 5 minutes) in line with the excellent *in vitro* permeability. However, the poor metabolic stability was most likely the reason for the rapid clearance of **40** from the brain.

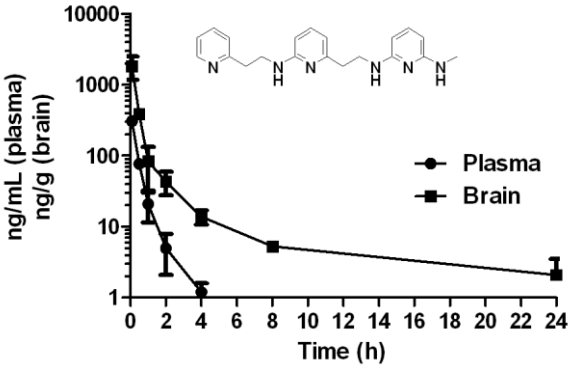


Figure 4. Brain and plasma levels of **40** after i.v. administration of 2 mg/kg

Table 1
Pharmacokinetic properties in male Swiss mice[a]

	40 (plasma)[b]	**40 (brain)**[b]
$t_{1/2}$ (h)	0.63 ± 0.18	5.23 ± 2.71
AUC (ng/mL* h)	150 ± 13	863 ± 195
V_d (L/kg)	11.99 ± 3.24	18.94 ± 12.93
C_{max} (ng/mL)	308 ± 7	1845 ± 484
B/P[c]	5.98 ± 1.51	

[a]Administration at 2 mg/kg i.v.
[b]iv formulation (100 % DMSO).
[c]B/P = brain to plasma ratio, 5 minutes post i.v dosing.

In summary, we have identified the 2,6-diaminopyridine moiety as a key component in the design of potent inhibitors of $A\beta_{1-42}$ aggregation with good aqueous solubility. The most balanced profile between inhibition of $A\beta_{1-42}$ aggregation and aqueous solubility was achieved by separating the 2,6-diaminopyridine unit via a C2-linker from the adjacent 2,6-disubstituted pyridine(s). At least three 2,6-disubstituted pyridine moieties containing 3 hydrogen bond donors and 3 hydrogen bond acceptors in the correct arrangement, *i.e.* compounds **39-41**, **44**, were required for the most potent inhibition of Aβ aggregation. Further efforts describing additional compounds with improved metabolic stability and pharmacokinetic properties are in progress.

Supplementary data

Supplementary (experimental procedures for the preparation of compounds **2-9**, **11**, **12**, **14**, **16**, **17-19**, **21-25**, **26-44**, *in vitro* fluorescence assay) data associated with this article can be found, in the online version.

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24. The pharmacokinetic study of **40** was conducted by Eurofins ADME Bioanalyses (France) using 42 male Swiss mice around 5 weeks old. The animals were fed on pellets. The pellets and tap water were given "ad libitum". During the experiments, the animals were fasted at least 8 hours before the dosing, but they had free access to water. Access to food was restored 4 hours after dosing. The administered dose of compound **40** was 2 mg/kg at a concentration of 2 mg/mL using DMSO as vehicle. Animals were anaesthetised with Isoflurane® using an anaesthetic system for administrations, at intermediary blood sampling times and at sacrifice.

Graphical Abstract

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Synthesis and structure-activity relationship of 2,6-disubstituted pyridine derivatives as inhibitors of β-amyloid-42 aggregation

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