From the Department of Biosciences and Nutrition
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Synthesis of estradiol mimetics and potential anti-Alzheimers agents

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Stockholm, 2010

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ISBN 978-91-7457-069-4

ABSTRACT

This thesis is composed of two different projects, both dealing with the synthesis part of drug development.

The first project is in the subject of organometallic chemistry and deals with method development for the synthesis of substances that could be used in estrogen replacement therapy. It presents the successful use of CuO as *co*-reagent in various Stille couplings of sterically hindered bi- and heterobiaryls, meant to function as mimetics of the estradiol backbone. The results clearly point to the advantages of the method compared to the classical Stille cross-coupling methodology for this kind of reactions.

The second project covers the synthesis of ligands designed to bind to the Aβ-peptide and keep it in α-helical form. These new synthesized ligands are peptoids consisting of four building block units. Synthesis of peptoids was evaluated both in solution and on solid phase. Several new building blocks for peptoids were synthesized including the new amino acid N^{γ}-(2-aminoethyl)-2,4-diaminobutanoic acid. The new ligands will be evaluated with respect to Aβ helix stabilisation and subsequent biological assays.

LIST OF PAPERS

I Synthesis of estradiol backbone mimetics *via* the Stille reaction using copper(II) oxide as co-reagent, *Tetrahedron Letters*, in press

II Synthesis of peptoid ligands for the helical conformation of the Alzheimer's disease related amyloid β-peptide (manuscript)

CONTENTS

LIST OF ABBREVIATIONS

Aβ	amyloid β-peptide
AD	Alzheimer's disease
Boc	*tert*-butoxycarbonyl
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
Cbz	carbonylbenzyloxy
DBU	1,8-diazobicyclo[5,4,0]-undec-7-ene
DIPEA	diisopropylethylamine
DMAP	dimethylaminopyridine
DMF	*N*, *N*-dimethylformamide
DMSO	dimethylsulphoxide
EDT	ethanedithiol
ES-TOF .	electron spray – time of flight (mass spectrometry)
Fmoc	fluorenylmethyloxycarbonyl
HATU	(2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluoro phosphate
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOAc	acetic acid
HPLC	High performance liquid chromatography
MeOH	methanol
MS	mass spectrometry
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
OBn	bensyloxy
Pfp	pentafluorophenyl
Pbf	2,2,4,6,7-pentamethyldi-hydrobenzofuran-5-sulfonyl
RP-HPLC	reverse phase - HPLC
Succ	succinyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIS	triisopropylsilane
TLC	thin layer chromatography

Amino acids used
(natural and non natural)

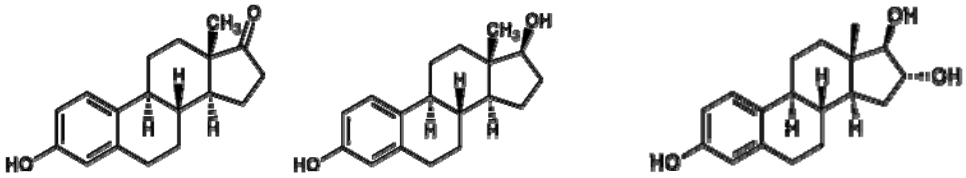
Arg	arginine
Dab	2,4-diaminobutanoic acid
Dap	2,4-diaminopropanoic acid
Glu	glutamic acid
Gla	glutaric acid
Lys	lysine
Orn	ornithine
Trp	tryptophane

Method development for synthesis of Estradiol mimics

Background

Biology

Estrogen is a collective name for a group of steroid compounds, named for their importance in the estrous cycle. It consists of estriol, estrone and estradiol (**Figure 1**). Out of these, estradiol is the predominant form in non-pregnant females. Estrogens play an important role in reproductive, skeletal, cardiovascular, and central nervous system function in both males and females.



Estrone **Estradiol** **Estriol**

Figure 1. Estrogens

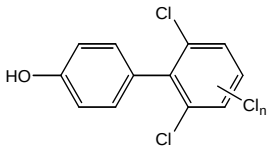
Post-menopause estrogen production decrease is associated with several pathological processes like osteoporosis, coronary heart disease and Alzheimer's disease. These diseases are usually treated by replacing the loss of the bodies own estrogen production with exogenous estrogen, so called estrogen replacement therapy (ERT). However, although ERT replaces lost estrogen levels, it may also cause breast cancer. [1] Therefore it is important to find alternative compounds that stimulate estrogen action only in target organs, certain cell types or in receptor isoforms. These properties are found in selective estrogen receptor modulators (SERM's).

SERM's act as agonists in tissues where it is needed, like for instance in bone tissue, while at the same time as antagonists in such tissues like uterus, breast and brain. In this way they can arrest osteoporosis by stimulating

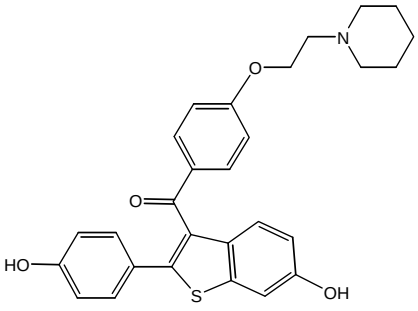
continued bone turnover while, for example, simultaneously inhibiting cancer progression in uterus and breast tissue.[2] Although fairly effective in arresting side effects, even SERM's have been shown to be a source of side effects like thrombosis[1] and increases in endometrial and breast cancer. Many SERM's also, suffer from low bioavailability, they are not absorbed very well by the body and well in the system they are quickly metabolized and lose most of their effect.[1] Therefore it is of importance to continue drug discovery in this field.

Chemistry

It has been observed that a wide variety of compounds can exhibit estrogenic activity, *e.g.* the naturally occurring phytoestrogens and various synthetic xenoestrogens. In a study of polychlorinated biphenyls (PCB's) by Korach in 1988[3], it was found that most of them showed estrogenic activity. The ones with the highest activity were the conformationally restricted 4'-hydroxy PCB's, which are those with one or two chlorines *ortho* to the biphenyl bridge (**Figure 2**). LeSuisse *et al*, 13 years later, synthesized several analogues to these using the Suzuki[4], Negishi[4] and Ullman[5] couplings with several variations.[6] Yields were low, especially when the halogen cross-coupling partner was substituted at both *ortho* positions.[3] A viable alternative for the assembly of sterically hindered biaryls in reasonable yields was to use CuO as *co*-reagent, as described by the Salo Gronowitz group.[7]



Di-ortho-substituted PCB



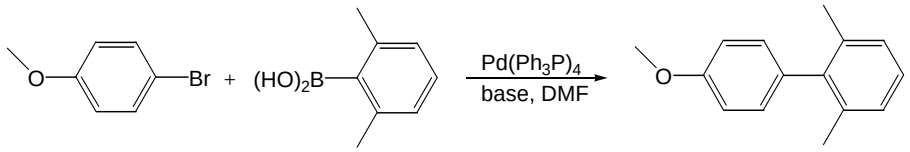
Raloxifen

Figure 2. Xenoestrogens.

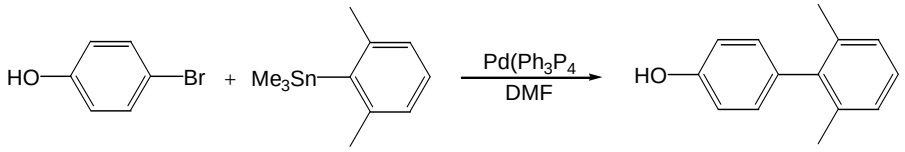
The couplings

The Suzuki coupling has emerged as the most common coupling for the preparation of biaryl compounds during the last decade, partially due to the inherent toxic properties of organotin reagents used in the alternative Stille coupling. In **Scheme 1** below, the Suzuki and the Stille couplings are shown. The Suzuki-coupling uses boronic acids and aryl halogens as coupling partners, while the Stille coupling uses stannanes and aryl halogens. Furthermore, it can be noted that the Stille-coupling requires no protection for its phenol moiety, as the reaction conditions for the Stille coupling are non-aqueous and neutral. Protection could therefore be crucial for the Suzuki coupling.

Suzuki coupling



Stille coupling

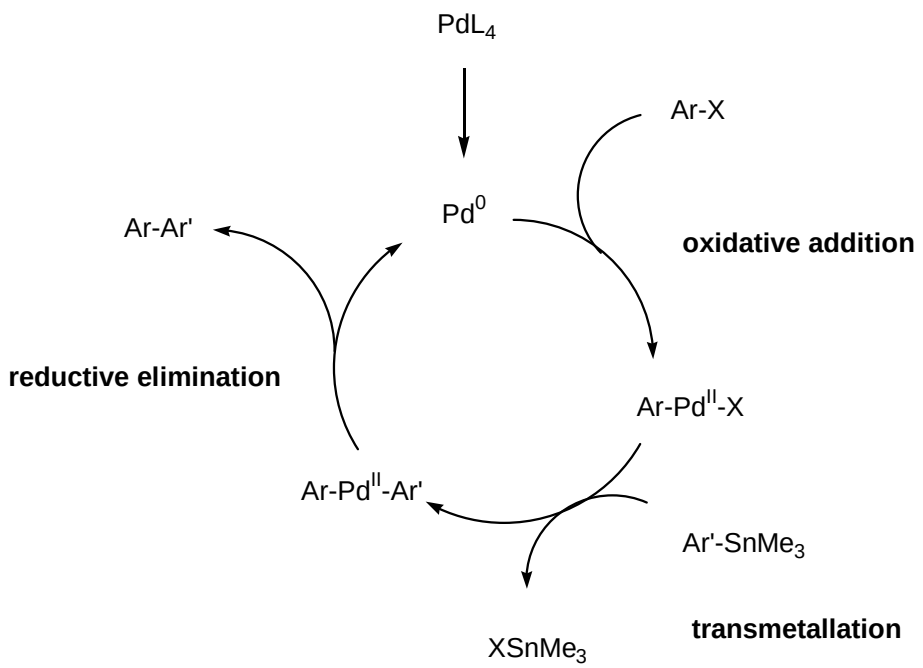


Scheme 1. Comparison of reaction conditions used in the Suzuki coupling and the Stille coupling respectively.

Initial studies in synthesis of aryl thiophene backbones with Suzuki coupling gave poor results (unpublished data) and in light of this we decided to investigate the feasibility of the Stille coupling for the synthesis of estradiol mimetics. Stannanes also have longer shelf life than its boronic acid counterparts. Organotin compounds can be toxic, both for man and nature,

but since Renaud *et al*'s report 1998[8], we now know that all traces of tin in a reaction mixture can be completely removed upon work-up in the presence of Et_3Al or NaOH (aq).

The Stille cross-coupling reaction is a palladium–catalyzed reaction. The use of palladium as a catalyst means that it is recycled as depicted in **Scheme 2** below.



Scheme 2. Palladium-catalyzed Stille cross-coupling reaction.

A range of metal salts and oxides have been added to the reaction as *co*-reagents in order to see whether they could catalyze the reaction and a few have stood out more than others.[7b] CuO gave the best results catalyzing cross-couplings of sterically hindered reaction partners.

The mechanistic role of CuO is still uncertain. We can only note that sluggish Stille cross-coupling reactions can be accelerated by CuO addition, and that it is generally accepted that the rate determining step is the transmetallation

step. What this means mechanistically still needs more research to say for certain.

As a conclusive assessment of the role of Cu has yet to be made, one can only say that even though 18 years have passed since the discovery of the accelerating effect of amphoteric oxides as CuO and Ag_2O in the Stille-coupling, no viable mechanism can still explain its effect.

Ligandless palladium

We also wanted to try to run our reactions using another palladium strategy, namely "ligandless" palladium,[9, 10] as it has been reported to show good yields in systems similar to our own. Ligandless palladium means adding a palladium species with more loosely complexed ligands and then exchanging those ligands *in situ* with another type of ligand. Several types of palladium-ligand complexes were considered, but the one that seemed best suited for our reaction was the loosely complexed palladium species tris(benzylideneacetone)dipalladium (Pd_2dba_3) and as the ligand exchanged for trifurylphosphine was chosen (Figure 3), as it reportedly had produced good yields in reaction conditions similar to ours.[11] Also, our group has previously attempted using the more loosely binding ligand $AsPh_3$ in similar reactions without success.[7d]

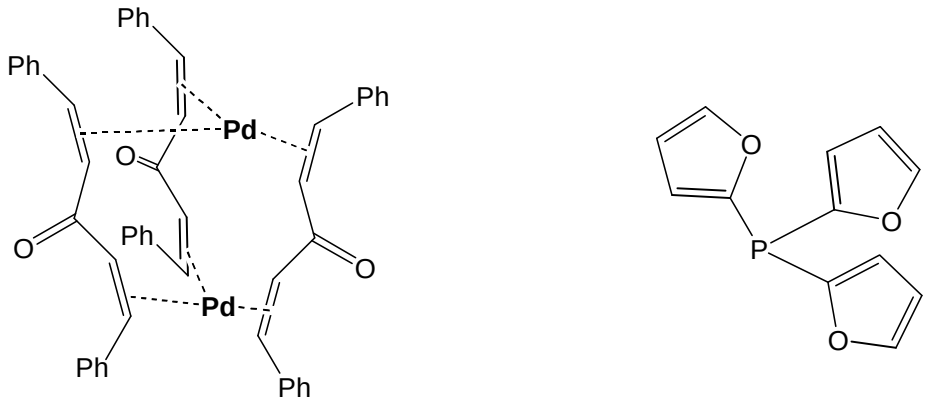
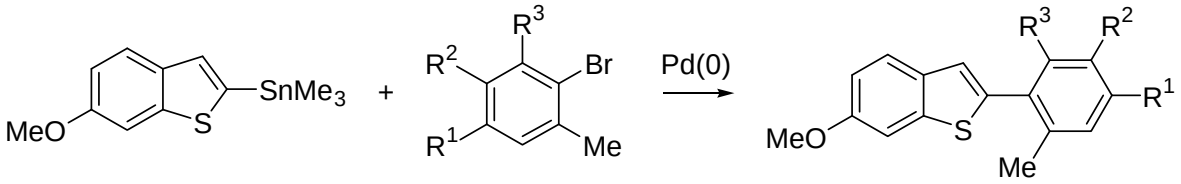


Figure 3. Tris(benzylideneacetone)dipalladium (Pd_2dba_3) and trifuryl-phosphine, respectively.

Results and discussion

In our paper "*Synthesis of estradiol backbone mimetics via the Stille reaction using copper(II) oxide as co-reagent*"[12] we have evaluated the use of CuO as *co*-reagent in the synthesis of xenoestrogen backbones based on hindered biphenyls and aryl thiophenes. First attempts were made to synthesize a starting building block for Raloxifen by coupling 2-trimethylstannyl-6-methoxybenzo[b] thiophene with substituted phenyl bromides. The reactions were made as follows (Method A): the stannane was added *via* a syringe to a stirred mixture of the appropriate bromophenol in DMF containing tetrakis(triphenylphosphine) palladium(0) (5 mol%), and with or without the presence of CuO (equimolar amounts). Prior to stannane addition, the mixture had been purged with argon for 15 minutes. The reactions were heated at 95-100 °C over night.



Entry	R^1	R^2	R^3	Yield without CuO (%)[a]	Yield with CuO (%)[a]
1	H	H	H	n.d.	98
2	H	H	Me	0	61
3	OH	H	Me	0	37
4	OH	Me	H	n.d.	46

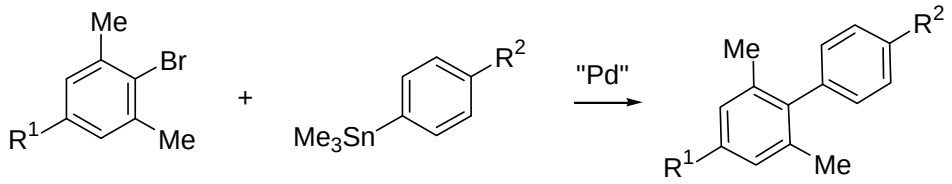
Table 1. Palladium-catalyzed cross-coupling of 2-trimethylstannyl-6-methoxybenzo[b]thiophene with aryl bromides. [a] Yields refer to isolated products. n.d. = not determined.

As can be seen, the presence of CuO was mandatory in order to obtain the desired product (entries 2 and 3). Yields were lower when the R^1-position was substituted with a hydroxyl group.

Boronic acids (Suzuki coupling) were also evaluated as coupling partners using a wide variety of conditions, but resulted in complex reaction mixtures and low yields of the desired products (unpublished data).

Next, we wished to investigate the formation of a similar kind of estrogenically active compounds, sterically hindered biaryls, 4-(1'-hydroxy-3',5'-dimethylphenyl)-benzaldehyde, with and without benzyl protection of the phenolic hydroxyl group (Table 2). The presence of the R^2-formyl group represents the synthetic precursor of the hydroxymethyl group in the estradiol mimetic shown in Figure 2. These reactions were performed both in the absence and in the presence of CuO (Table 2) under conditions identical to those for the aryl thiophene derivatives. The reactions were allowed to run until no further development could be observed (as seen by TLC). The reaction mixture was then refluxed with ethyl acetate and activated carbon, filtered, evaporated and analyzed by HPLC. The results from these reactions are shown below (Table 2), conversions were determined by HPLC using an internal standard[13].

For this second kind of biaryl, an alternative to the stronger binding triphenylphosphine palladium ligands of the first method was also to be evaluated in the cross-coupling. These reactions were run under ligandless conditions (Method B): 4-trimethyl-stannylbenzaldehyde was added to a reaction mixture of the bromide and Pd_2Dba_3 (5 mol%) and trifurylphosphine in DMF with heating set to 65°C (the mixture had been purged with argon prior to the bromide addition). The "ligandless" reactions were evaluated as stated for the reactions with the tetrakis(triphenylphosphine) ligands.



Entry	R^1	R^2	Method	Reaction time, h [a]	Yield, % [a]
1	OH	CHO	A	20 (102)	94, 80 [b] (51)
2	OBn	CHO	A	18 (23)	47 (45)
3	OH	CHO	B	69 (30)	25 (5)
4	OBn	CHO	B	96 (50)	8 (9)
5	OH	COOH	A	28 (20)	n.d. (n.d.)[c]

Table 2. Palladium-catalyzed cross-coupling of arylstannanes with arylbromides. [a] Yields refer to conversions determined by HPLC analysis (unless otherwise stated) and yields and reaction times in brackets refer to runs without CuO. [b] Isolated yield in a separate synthesis. [c] reactions gave complex mixtures and the desired product was not isolated or identified. n.d. = not determined.

The first observation is that also in the couplings with benzaldehyde stannane (using Method A), addition of CuO results in better yields and shorter reaction times. Although, with the benzyl protected bromophenols there was virtually no effect of CuO addition. The reason for running the coupling reactions under argon atmosphere was that the benzaldehyde stannane turned out to be extremely sensitive to air oxidation. Merely leaving the benzaldehyde stannane exposed to air was enough to oxidize it completely in a couple of weeks. After this discovery we found ourselves in the possession of the corresponding benzoic acid stannane and it was natural to include it in our study. However, the reaction with this substrate turned out to result in complex mixtures.

For the runs employing ligandless conditions the yields from the couplings in were considerably lower, compared to those from Method A, irrespective of use of a protected or unprotected phenolic function of the stannane cross-coupling partner. However, just like in Method A, yields were higher in the presence of CuO in the reaction with the unprotected phenol as cross-coupling partner. As opposed to with Method A the reaction times were higher when CuO was used as *co*-reagent.

Comparing the two methods, a few observations can be made. For the biaryl couplings, the use of stronger binding ligands (Method A) was clearly the better alternative, irrespective of whether CuO was used as co-reagent or not. The palladium-catalyzed cross-coupling, was considerably more effective in combination with CuO than without (Tables 1 and 2) when an unprotected bromophenol was used. It resulted in higher yields as well as a substantially shorter reaction time in the reaction with the unprotected phenol (Table 2, entry 1), and with the benzothiophenes there was no detectable product whatsoever without CuO-addition. When a protected phenol (-OBn) was used as the coupling partner, CuO-addition did not give much improvement (entry 2, Table 2). Ligandless conditions (Method B) gave quite inefficient cross-coupling of these substances, for both unprotected and protected phenols (Table 2, entries 3 and 4), both with and without CuO.

Conclusion

In summary, we have shown that the Stille palladium-catalyzed cross-coupling of benzothiophene and benzaldehyde stannanes with bromophenols is greatly improved when the co-reagent CuO is used. Comparing the two Methods A and B, also suggests the use of tetrakis(triphenylphosphine) palladium(0) rather than "ligandless" conditions for Stille cross-coupling reactions of hindered biaryls.

Peptoid ligands for the Alzheimer Aβ-peptide

Background

Biology

Many diseases are caused by misfolded peptides and proteins, despite the many control mechanisms nature provides to insure proper folding.[14] Around 40 (and increasing) diseases are associated with the misfolding and aggregation of proteins and peptides into structures known as amyloid fibrils.[15] This group includes, for example, the neurodegenerative Alzheimer´s, Parkinson´s and Creutzfeld-Jacob´s diseases.[16]

Alzheimer's disease (AD) is a progressive brain disorder that gradually destroys a person's memory and ability to learn, reason, make judgments, communicate and carry out daily activities. It is the most common cause of dementia, accounting for about 60% of the cases[17] and has historically been strongly associated with amyloid plaque. Amyloid plaques are composed of amyloid fibrils assembled into disordered networks, and the amyloid fibrils, in turn, are composed of polypeptide chains in β-strand confirmation, which form β-sheets running perpendicular to the long axis of the fibril. These structures share the same morphology regardless of what peptide they are formed from. They are also protease resistant and the organism is unable to degrade them to any significant extent.

Previously it was thought that the Alzheimers diseases related amyloid plaques in brain caused the neurotoxicity. There is, however, increasing evidence that the neurotoxicity is mainly caused by soluble intermediates or even by the aggregation process.[18]

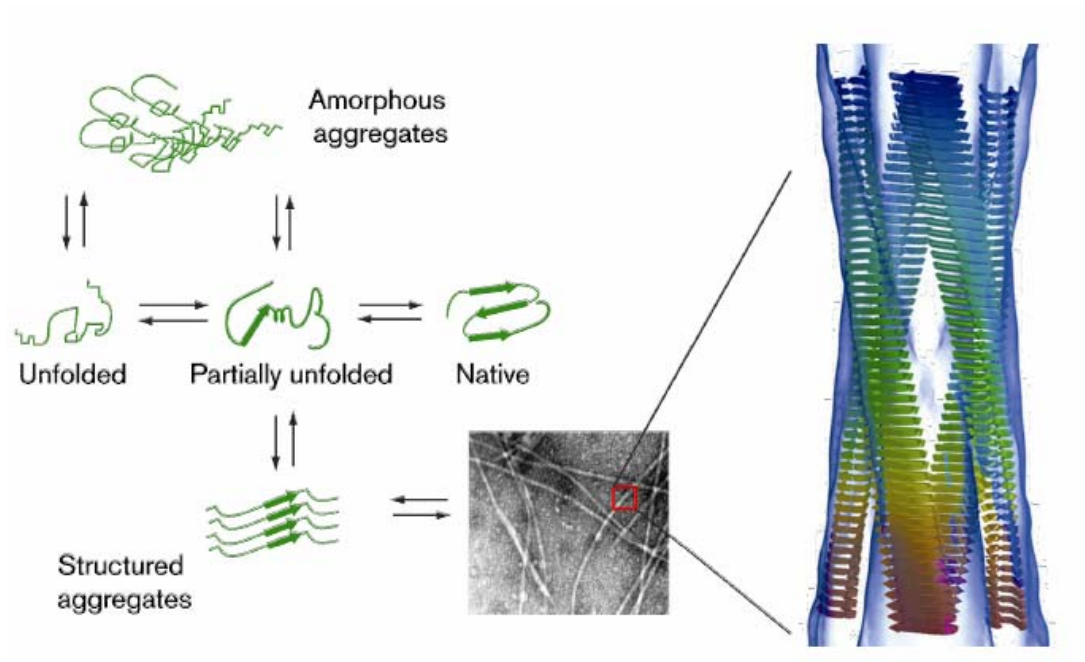


Figure 3. Protein folding and misfolding. Aβ-peptide in its various forms, from unfolded to amyloid fibrils. (Fibril model adapted from Jimenez *et al.*, 1999)

It should be mentioned that amyloid plaques in general are not only pathological in nature. They can also be a part of a wide range of natural functions in living organisms.[19]

In the case of Alzheimer's disease, amyloid plack and all intermediates are mainly formed from the same building block, namely the amyloid-β peptide (Aβ-peptide). This is a part of a transmembrane peptide called amyloid-β precursor protein (APP). APP is expressed in a large variety of cell types throughout the body and has no known function.[23c]

The Aβ-peptide is formed as parts of APP are cleaved off. First a large segment of APP is cleaved off, leaving one part on the outside and the other on the inside of the membrane.

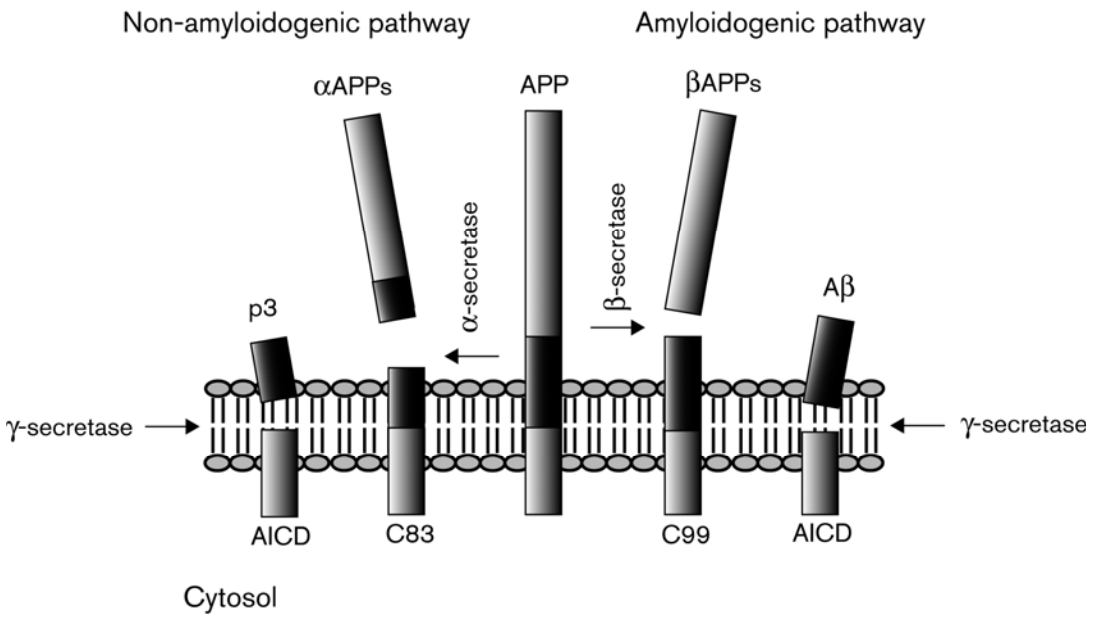


Figure 4. Formation of Aβ.

After β-secretase has cleaved off βAPP_s, Aβ is positioned partly in the membrane and partly on the outside as depicted in **Figure 5** below. As can be seen, the Aβ-peptide harbours two α-helices, one in the membrane and one directly outside the membrane.

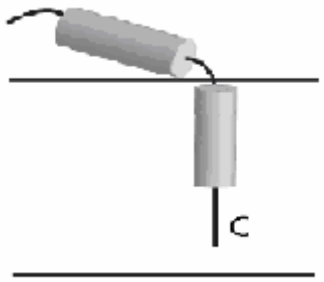


Figure 5. Aβ-peptide after β-secretase has cleaved off the outermost segment.

The resulting peptide is cleaved once more, this time by γ-secretase, forming the Aβ-peptide. When departing from the membrane it loses its helical conformation and turns into other forms with toxic properties, either as a soluble monomer of various conformations, or forming soluble oligomers or aggregates of these (*see above*, **Picture 3**).

The Aβ–peptide contains a discordant helix (residue 16-23) i.e. a helix composed of amino acids with a high propensity for β-strand conformation. Peptides derived from this discordant helix region form fibrils and, in Aβ, this region has been found essential for fibril formation[20] The aggregation process of Aβ is not fully understood and it is unclear which forms of Aβ that are toxic. Early aggregates are not structurally defined, making inhibitor design a difficult task and it is also possible that targeting species on the fibrillation pathway may result in accumulation of toxic oligomers. A more appealing idea would be to target and stabilize an Aβ-monomer, thereby preventing misfolding and subsequent amyloid formation.

We have previously shown that by using small designed ligands, directed towards the discordant region of Aβ (residues 13-23), it is possible to stabilize the helical structure of a freshly cleaved off Aβ-peptide, thereby reducing aggregation *in vitro.* [21] These ligands also reduced cell toxicity of $A\beta_{1-42}$ and prevented $A\beta_{1-42}$ induced reduction of γ oscillations of hippocampal slices. Gamma oscillations play an important role in higher processes in the brain, such as learning, memory, cognition, and perception[22] and are markedly reduced in patients diagnosed with Alzheimer's disease[23]
In addition, oral administration of two of these compounds in the *Drosophila* model of Alzheimer's disease[24] increases longevity, decreases locomotor dysfunction and reduces neuronal damage[25] Altogether, these results are promising and suggest that this concept is worth pursuing and may lead to an effective Alzheimer's treatment.

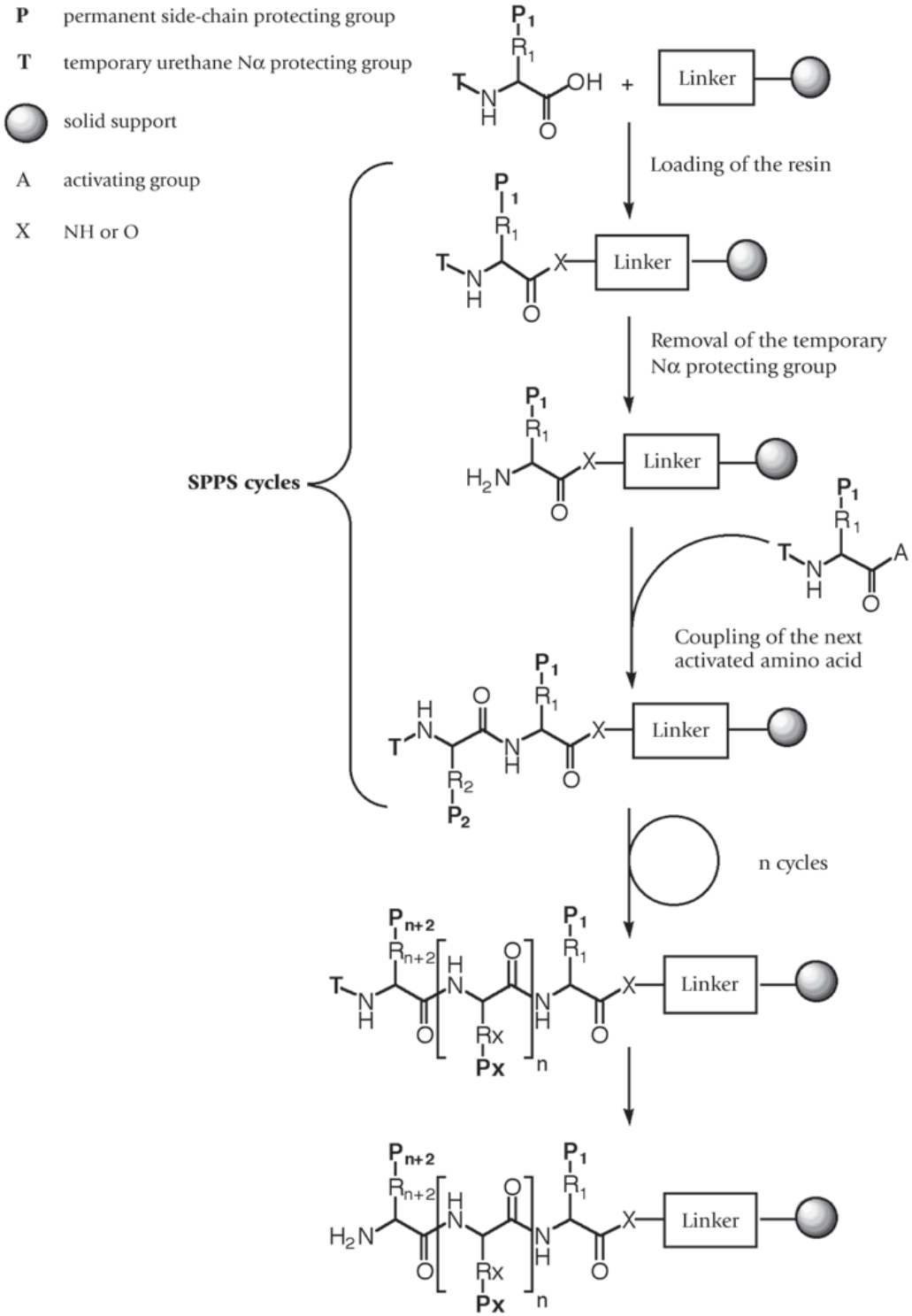
Chemistry

To further test our hypothesis and to develop ligands with higher affinity to the Aβ region covering residues 13-23, several new peptoid ligands designed to bind and stabilize the Alzheimer's disease related amyloid β-peptide in α-helical conformation were synthesized. In addition, we explored different methodologies to diversify the synthesis of potential ligands.

Peptide synthesis

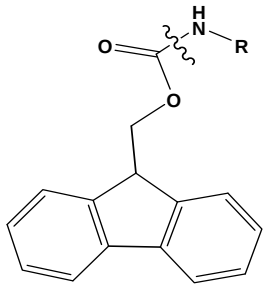
Peptide synthesis in solution phase is commonly used and sometimes still preferred, but it is often tedious and time consuming, especially when longer peptides are to be made. Already in 1963, Bruce Merrifield reported successfully having synthesized a peptide employing a solid phase scheme. He had attached the starting amino acids to small polystyrene beads, and then in a stepwise fashion coupled further amino acids, forming a peptide that was subsequently cleaved off and isolated. This was no less than a revolution in peptide synthesis as it greatly could improve synthesis of longer peptides and in the same time introduced a protocol that could be robotized. The protocol (**Scheme 3**) begins with the swelling of the resin in order to open up the pores, thereby increasing the surface available. The starting amino acid is attached to the resin, after which it is washed and deprotected. The amino acid to be attached, either has an activated carboxyl group or a condensing agent, like the today most commonly used HBTU or HATU reagents (**Figure 6**), for the coupling. The deprotection of a previously blocked amino group, leaves it ready to couple to the next amino acid. This cycle is then repeated, starting with the coupling of the next amino acid. When all amino acids have been coupled, the resulting peptide is cleaved off from the resin and isolated.

An advantage of solid phase peptide synthesis is that all the time consuming isolation steps between couplings can be cut out. Solid phase peptide synthesis usually follows a standardized protocol (exemplified in **Scheme 3**).

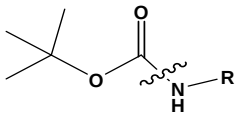


Scheme 3. Solid phase peptide synthesis.

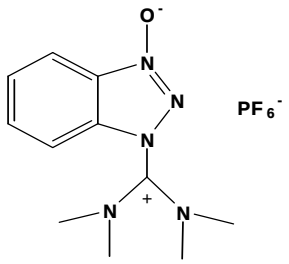
Commonly all functionalities of the amino acids that could disturb the reaction are blocked by protecting groups. With several active groups on the amino acids, protecting groups play a central role in the synthesis of peptides and peptoids. Today there exists a great amount of various protective groups for all the side chain functionalities that has to be blocked from taking part in a reaction, when they are not meant to. Also it is important to be able to selectively cleave only certain protecting groups and when there are many different functionalities that need protection, so called orthogonal protecting groups are useful. This means protecting groups that are cleaved in different conditions, to be able to selectively cleave the desired groups. Two of the most frequently used protecting groups are the Fmoc-group and the Boc-group, standing for fluorenylmethyloxycarbonyl and *t*-butoxycarbonyl respectively (**Figure 6**).



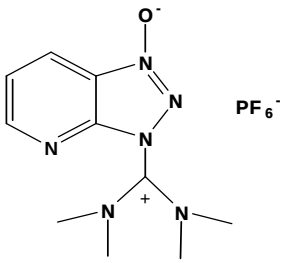
Fmoc



Boc



HBTU



HATU

Figure 6. Protecting groups and condensing agents.

The Fmoc-group is easily cleaved off under basic conditions, using a 20% piperidine in DMF-solution, whereas the Boc-group is cleaved under acidic conditions (typically TFA containing solutions).

Results and discussion

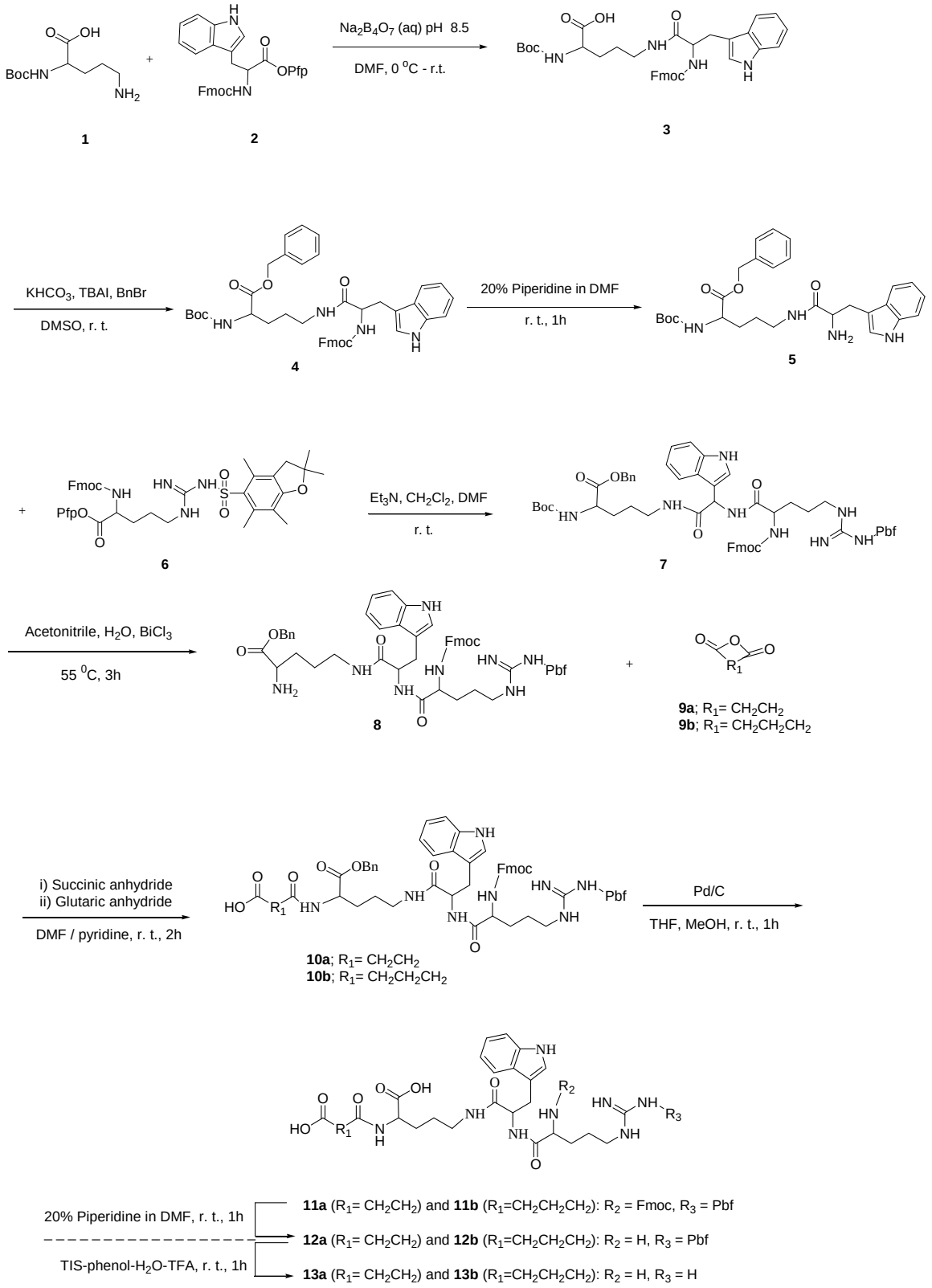
Different peptoids for binding to and stabilizing Aβ-peptide in α-helical conformation were synthesized. Synthesis methods were evaluated and two peptoids were isolated from solution synthesis and one, containing a novel amino acid, was isolated from solid support synthesis.

For the solution synthesis (**Scheme 4**), we started with the synthesis of the dipeptoid Orn(Trp) with the side chain amino group of ornithine forming the amide bond with tryptophane, instead of, as with peptides, the α-amino group. We wanted to have this as a building block in order to avoid the risk of cyclization, which could occur after deprotection of the γ-amino Fmoc-group of Orn following coupling to the diacid. Fmoc based synthesis of a peptoid where one intermediate is an esterified diaminobutyric acid (Dab) with a free γ-amino group, could potentially give intramolecular cyclisation which would truncate further couplings.

In the first step the pentafluorophenyl ester of Fmoc-D-Trp (**2**) was coupled to the δ-amino group of Boc-D-Orn-OH to give **3**. We then tried to make the *t*-butylester of **3**, *via* a published methodology[26] but yields were low, so it was instead protected as a benzyl ester (**4**) using benzyl bromide[27] in good yield. The Fmoc group was then removed and the resulting compound **5** was coupled to Fmoc-Arg(Pbf)OPfp **6** to give **7** in 88% yield.

In order to minimize side reactions on tryptophane while keeping the Pfp protection intact, we tried removal of the Boc group using $BiCl_3$ in the presence of H_2O.[28] The literature procedure gave 10% deprotection or less,

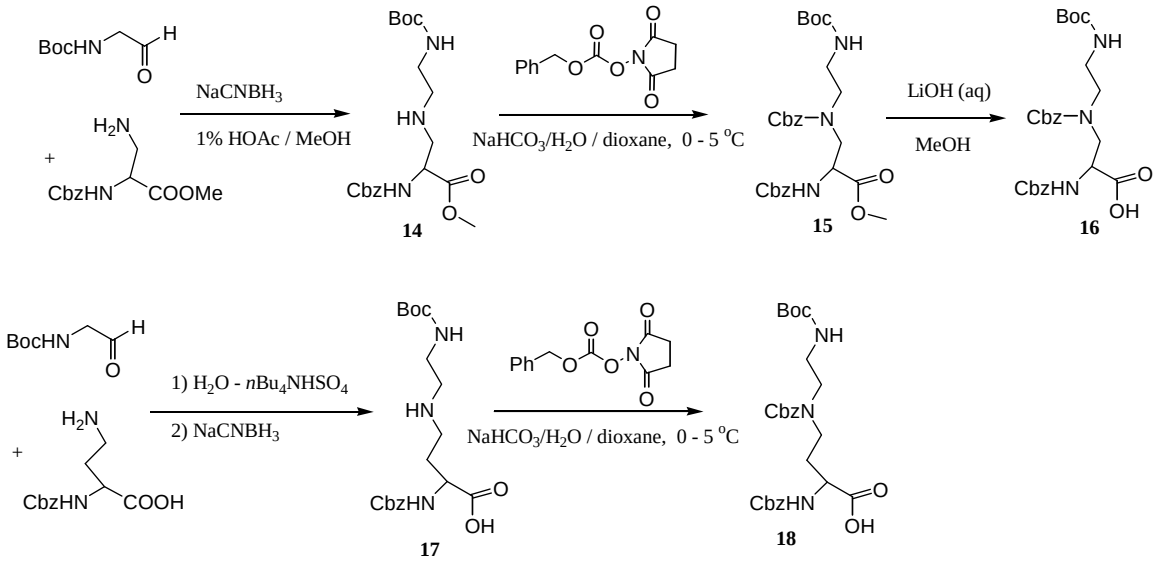
but after alteration of the protocol ($BiCl_3$ ~ 3 eq) the desired peptoid **8** was achieved in 51% yield. **8** was then coupled with either succinic anhydride or glutaric anhydride to give the desired peptoids **10a** and **10b** respectively. After removal of the benzyl group by catalytic hydrogenation using Pd/C, we arrived at **11a** and **11b**. This was followed by removing Fmoc (20% piperidine in DMF) giving **12a** and **12b**, and then the final deprotection by a TFA-based cocktail (Phenol: EDT: thioanisole: H_2O: TFA), containing scavengers to minimize modification of Trp residues[29] The desired product was however impure and hard to purify. Therefore we instead attempted deprotection of **12a** and **12b** using a TIS-TFA (Triisopropylsilane-phenol-H_2O-TFA) based cocktail[30] This resulted in purer material and **13a** and **13b** could readily be isolated by semi preparative RP-HPLC.



Scheme 4. Solution phase synthesis of peptoids **13a** and **13b**.

Synthesis of peptoids in solution is rather time consuming so we decided to investigate whether a solid support approach could be used. This way time consuming intermediate isolation steps could be completely omitted. It may be necessary to decrease the risk of cyclization of the Orn and Dab amino acids by attachment of a D-Trp-Orn/Dab dimer as one unit to the support. But it would also be interesting to see whether the feared cyclisation would be sufficiently slow to allow synthesis in a stepwise fashion.

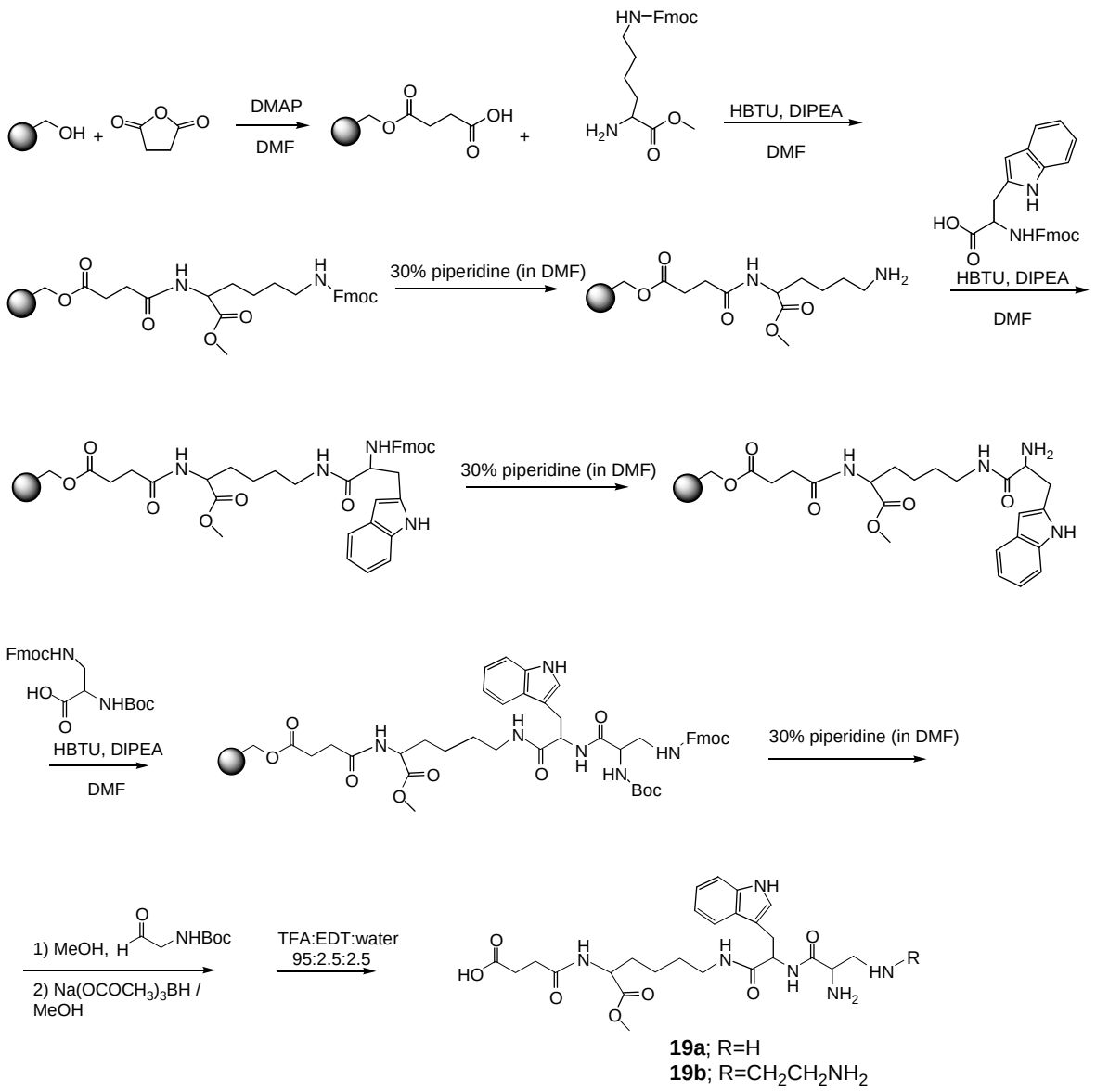
We also wanted to incorporate artificial triamino acids in the position occupied by arginine in the peptoid structures described above. For this protected derivatives of N^{β}-(2-aminoethyl)diaminopropionic acid[31] and the novel amino acid N^{γ}-(2-aminoethyl)diaminobutyric acid were synthesized (Scheme 5). The central synthesis step for both non-natural amino acids, although carried out slightly differently for each one, was the coupling of diamino acid to N-Boc-glycinaldehyde, followed by reductive amination using $NaCNBH_3$. The N^{β}-(2-aminoethyl)diaminopropionic derivative **14** was synthesized by portionwise addition of $NaCNBH_3$ to a chilled solution of methyl N^2-benzyloxycarbonyl-2,3-diaminopropionic acid hydrochloride and N^2-*(tert*-butoxycarbonyl)glycinal in 1% acetic acid in methanol. Subsequent Cbz-protection gave the ester **15**, which was then hydrolyzed to **16** with LiOH. For the novel triamino acid derivative, N^{γ}-(N-*tert*-butoxycarbonyl-2-aminoethyl)-N^{α}-benzyloxycarbonyl-2,4-diaminobutanoic acid, the methodology used for the reductive amination was changed as we used the acid instead of the ester derivative. The solubility of the starting material was poor, even after addition of DMF, so the reaction was performed in an aqueous solution of Bu_4NHSO_4, resulting in **17**. This derivative was then additionally Cbz-protected using benzyloxycarbonylsuccinimide to give **18**.



Scheme 5. Synthesis of non-natural triamino acid building blocks **16** and **18**.

Before using the synthesized triamino acids in synthesis of peptoids, we decided to test an alternative route where, instead of using the synthesized bulding blocks in solid phase synthesis, the modified amino acid would instead be formed "in situ" on by performing the reductive amination on the support (**Scheme 6)**.

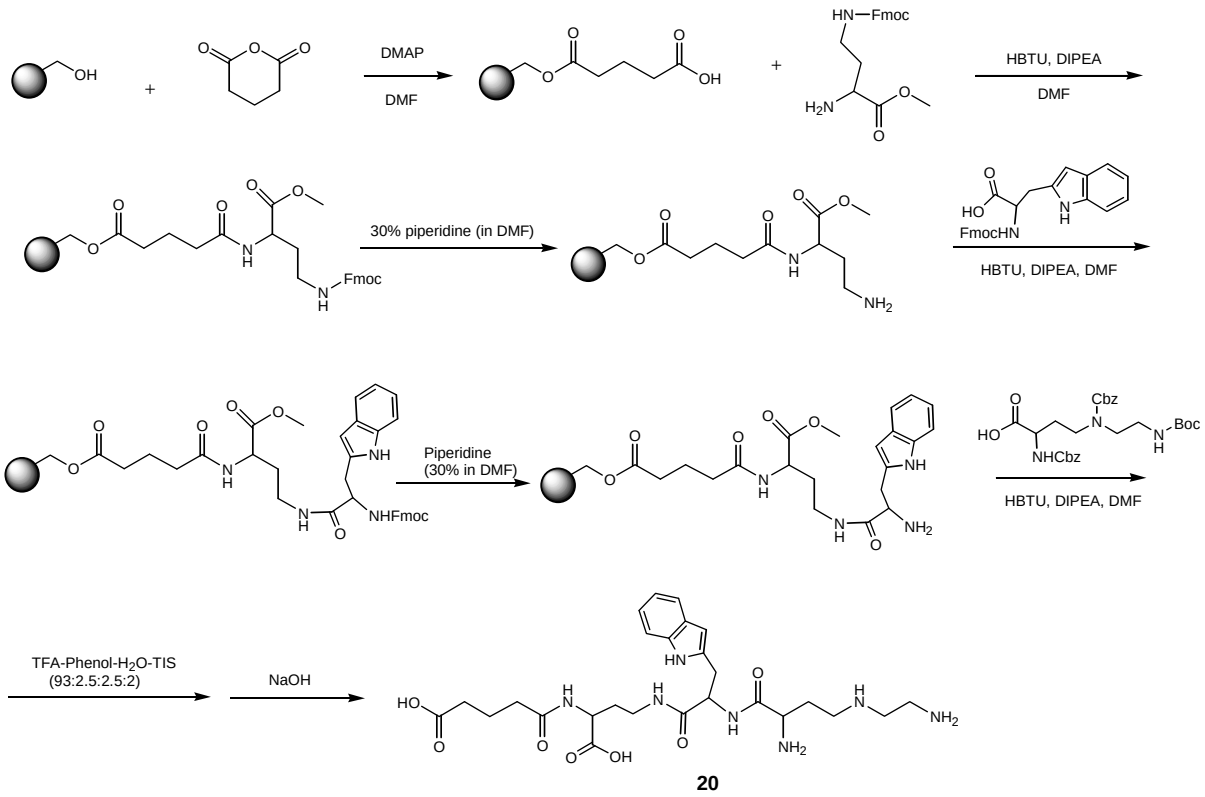
For this solid phase synthesis, the support (Wang polystyrene resin) was first reacted with succinic anhydride, then H-Lys(Fmoc)OMe was attached to the succinate support. Subsequent steps of Fmoc deprotection and coupling with D-Trp and then a diaminopropionic acid derivative were performed to give solid supported N^{α}-Succinyl-Lys-N^{ε}-((N^{α}-Boc-N^{β}-Fmoc-Dap)-D-Trp)OMe. After removal of Fmoc from the side chain of Dap, the other part of the artificial amino acid was attached by reductive amination with N-Boc-glycinal and $Na(OCOCH_3)_3BH$. After cleavage from support, analysis of the crude material by MS showed that the desired product **19b** had been obtained, but also that the main product was **19a** which is the peptoid product expected if the reductive amination step failed.



Scheme 6. Attempted solid phase synthesis of tetrapeptoid **19b** by reductive amination on support.

Going back to our original thoughts, we decided to make a peptoid on solid support and to include the novel triamino acid building block **18** in the synthesis. This synthesis was initiated by functionalizing the Wang support with glutaric anhydride, which was in turn connected to Dab(Fmoc)OMe. After deprotection of the the γ-amino group of the Dab residue, Fmoc-

protected D-Trp was attached. The Fmoc on the D-Trp was then removed and the new triamino acid building block **18** was coupled to the α-amino of the D-Trp residue using HATU as condensing agent. After cleavage from support by a TFA/TIS/Phenol cocktail and subsequent cleavage of the methyl esther by NaOH, the peptoid **20** containing the new amino acid N^{γ}-aminoethyl-2,4-diaminobutanoic acid could be isolated as the main product by RP-HPLC.



Scheme 4. Solid phase synthesis of tetrapeptoid **20** on Wang resin.

CONCLUSION

This project has produced several new potential ligands for stabilizing the α-helical conformation of the Alzheimer's disease related Aβ-peptide. The peptoid ligands were synthesized both by synthesis and on solid support. In addition a novel amino acid N^{γ}-aminoethyl-2.4-diaminobutanoic acid has been synthesized and incorporated into one of these peptoids.

Furthermore, a couple of solid phase procedures have been investigated. Reductive amination on support for attachment of the non-natural triamino acids was attempted, but was quite inefficient. Although the method could be optimized, it seems more reliable and straightforward to do the synthesis by attaching the triamino acids as one pre-prepared building-block.

Our reactions did not seem to suffer much from the intramolecular cyclization reaction of the deprotected esterified diaminobutyric acid that we had initially feared. If it does happen during the synthesis, it is clearly slow enough to allow the coupling to proceed nearly unrestricted, to give the desired peptoid as the main product. We can not exclude that the cyclisation occurred to some extent, but it is clearly not a major problem. This opens up for more ready production of libraries where the different parts of the peptoid, that interact with the Aβ-peptide, can be optimized. The synthesized peptoids as well as additional peptoids made by the solid phase approach will be investigated with respect to interaction with and retardation of aggregation of the Aβ-peptide.

AKNOWLEDGEMENTS

Thank you,

First and foremost my supervisors,
Roger, for allowing me to start in your group so that I could finish off the old skeleton in my closet that the estradiol project was, and for all your help and tutelage.
And equally as much, Johan Malm, for believing in me that I could start up this project once again and standing behind me.

Partha the Indian gentleman, for being a great collegue and teacher and driving the project forward.

Everyone else in the group, Dmytro, Malgorzata, Merita and Esther for all your help and the good times we have shared.

Our cooperation group in Uppsala, Jan Johansson for good collaboration, Charlotte for writing my bible on Aβ and Hanna and Jenny for a fun time in Hemavan.

Dr. Ivan Romero, amigo, for all your help and support and for all our good times together throughout the years.

Stefan, buddy, snubben, my man, for all our good times and enlightened discussions. Also thank you for your help and insights. Samhadi-boys forever!

Glenn Condie, my partner in crime. Two nutty souls came together and brought the world some more nuttiness.

Jeff and Ngarita, my great fika-buddies, for the ever interesting and entertaining moments we had together.

From the old times, Malin and Jealux for sharing our time together in the trenches and for shared laughs and anxieties, and Spiros for accepting me as a Phd-student.

The rest of JB's group, past and present.

And all our visitors, Joanna, the visitors from Mälardalens Högskola for a lot of laughs and good times, not to mention all our language lessons. I am probably fluent in several new languages by now.

All my crazy buddies outside the lab.

And last but not least,
Kicki, my sweetnes, for love and being by my side.

and

My family, for love and allways being there for me.

REFERENCES

[1] Reeves, G. K.; Beral, V.; Green, J.; Gathani, T.; Bull, D.; Abbott, S.; Baker, K.; Balkwill, A.; Bellenger, S.; Beral, V.; Black, J.; Brown, A.; Bull, D.; Casabonne, D.; Crossley, B.; Ewart, D.; Ewart, S.; Fletcher, L.; Gathani, T.; Gerrard, L.; Goodill, A.; Green, J.; Gray, W.; Hooley, J.; Kan, S. W.; Keene, C.; Langston, N.; Liu, B.; Nehushtan, S.; Pank, L.; Pirie, K.; Reeves, G.; Roddam, A.; Sherman, E.; Simmonds, M.; Spencer, E.; Stevens, R.; Strange, H.; Sweetland, S.; Taylor, A.; Timadjer, A.; Tipper, S.; Watson, J.; Williams, S., Hormonal therapy for menopause and breast-cancer risk by histological type: a cohort study and meta-analysis. *Lancet Oncol.* **2006,** 7, (11), 910-918.

[2] Dodge, J. A.; Lugar, C. W.; Cho, S.; Short, L. L.; Sato, M.; Yang, N. N.; Spangle, L. A.; Martin, M. J.; Phillips, D. L.; Glasebrook, A. L.; Osborne, J. J.; Frolik, C. A.; Bryant, H. U., Evaluation of the major metabolites of raloxifene as modulators of tissue selectivity. *J. Steroid Biochem. Mol. Biol.* **1997,** 61, (1/2), 97-106.

[3] Korach, K. S.; Sarver, P.; Chae, K.; McLachlan, J. A.; McKinney, J. D., Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. *Mol. Pharmacol,.* **1988,** 33, (1), 120-6.

[4] Ei-ichi Negishi, *Handbook of organopalladium chemistry for organic synthesis*, John Wiley & Sons, Inc**.,** New York, **2002**.

[5] Fanta, P. E., Ullmann synthesis of biaryls. *Synthesis* **1974**, (1), 9-21.

[6] Lesuisse, D.; Albert, E.; Bouchoux, F.; Cerede, E.; Lefrancois, J. M.; Levif, M. O.; Tessier, S.; Tric, B.; Teutsch, G., Biphenyls as surrogates of the steroidal backbone. Part 1. Synthesis and estrogen receptor affinity of an original series of polysubstituted biphenyls. *Bioorg. Med. Chem. Lett.,.* **2001,** 11, (13), 1709-1712.

[7] (a) Malm, J.; Bjoerk, P.; Gronowitz, S.; Hoernfeldt, A. B., Palladium-catalyzed coupling of heteroaryl alkylstannanes with heteroaryl halides in the presence of silver(I) oxide. *Tetrahedron Lett.,* **1992,** 33, (16), 2199-202. (b) Gronowitz, S.; Bjoerk, P.; Malm, J.; Hoernfeldt, A.-B., The effect of some additives on the Stille Pd0-catalyzed cross-coupling reaction. *J. Organomet. Chem.* **1993,** 460, (1), 127-9. (c) Malm, J.; Bjoerk, P.; Gronowitz, S.; Hoernfeldt, A.-B., Synthesis of benzo[c][2,7]naphthyridines by palladium-catalyzed coupling of pyridine methylstannanes with ortho bromoacetanilides in the presence of copper(II) oxide. *Tetrahedron Lett.* **1994,** 35, (19), 3195-6. (d) Bjoerk, P.; Malm, J.; Hoernfeldt, A.-B.; Gronowitz, S., Synthesis of various substituted benzo[c]-2,7-naphthyridines and an approach towards the skeleton of meridine. Considerations about the effect of copper(II) oxide. *Heterocycles,* **1997,** 44, 237-253.

[8] Renaud, P.; Lacote, E.; Quaranta, L., Alternative and mild procedures for the removal of organotin residues from reaction mixtures. *Tet. Lett.* **1998,** 39, (15), 2123-2126.

[9] Farina, V.; Krishnan, B., Large rate accelerations in the Stille reaction with tri-2-furylphosphine and triphenylarsine as palladium ligands: mechanistic and synthetic implications. *J. Am. Chem. Soc* **1991,** 113, (25), 9585-95.

[10] Beletskaya, I. P., The cross-couplingreactions of organic halides with organic derivtives of tin, mercury and copper catalyzed by palladium. *J. Organomet. Chem.* **1983**, (250), 551- 564.

[11] Clapham, B.; Sutherland, A. J., Stille Coupling Reactions of 4-Substituted 2,5-Diphenyloxazoles. *J. Org. Chem.* **2001,** 66, (26), 9033-9037.

[11] Nerelius, C.; Sandegren, A.; Sargsyan, H.; Raunak, R.; Leijonmarck, H.; Chatterjee, U.; Fisahn, A.; Imarisio, S.; Lomas, D. A.; Crowther, D. C.; Stromberg, R.; Johansson, J., alpha -Helix targeting reduces amyloid-beta peptide toxicity. *Proc. Natl. Acad. Sci. U. S. A.* **2009,** 106, (23), 9191-9196, S9191/1-S9191/13.

[12] Flöistrup, E. Goede, P.; Strömberg, R.; Malm, J., Synthesis of estradiol backbone mimetics via the Stille reaction using copper(II) oxide as co-reagent. *Tet. Lett.* **2010**.

[13] The response areas in the HPLC analysis (UV 260 nm) of the desired biaryl peak in the crude product and in the crude product spiked with a known amount of isolated biaryl compound was determined and the amount of biaryl compound in the crude product was determined from the difference between the spiked and non-spiked sample.

[14] (a) Meusser, B.; Hirsch, C.; Jarosch, E.; Sommer, T., ERAD: the long road to destruction. *Nat. Cell Biol.* **2005,** 7, (8), 766-772. (b) Kopito, R. R., ER quality control: the cytoplasmic connection. *Cell (Cambridge, Mass.)* **1997,** 88, (4), 427-430. (c) Hershko, A.; Ciechanover, A., The ubiquitin system. *Annu. Rev. Biochem.* **1998,** 67, 425-479.

[15] Chiti, F.; Dobson, C. M., Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **2006,** 75, 333-366.

[16] (a) Benson, M. D.; James, S.; Scott, K.; Liepnieks, J. J.; Kluve-Beckerman, B., Leukocyte chemotactic factor 2: A novel renal amyloid protein. *Kidney Int.* **2008,** 74, (2), 218-222. (b) Westermark, P.; Benson, M. D.; Buxbaum, J. N.; Cohen, A. S.; Frangione, B.; Ikeda, S.-I.; Masters, C. L.; Merlini, G.; Saraiva, M. J.; Sipe, J. D., A primer of amyloid nomenclature. *Amyloid* **2007,** 14, (3), 179-183.

[17] Fratiglioni, L.; Launer, L. J.; Andersen, K.; Breteler, M. M.; Copeland, J. R.; Dartigues, J. F.; Lobo, A.; Martinez-Lage, J.; Soininen, H.; Hofman, A., Incidence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. *Neurology* **2000,** 54, (11 Suppl 5), S10-5.

[18] (a) Sandberg, A.; Luheshi, L. M.; Sollvander, S.; Pereira de Barros, T.; Macao, B.; Knowles, T. P. J.; Biverstal, H.; Lendel, C.; Ekholm-Petterson, F.; Dubnovitsky, A.; Lannfelt, L.; Dobson, C. M.; Hard, T., Stabilization of neurotoxic Alzheimer amyloid-beta oligomers by protein engineering. *Proc. Natl. Acad. Sci. U. S. A* **2010,** 107, (35), 15595-15600, S15595/1-S15595/6.(b) Bitan, G.; Fradinger Erica, A.; Spring Sean, M.; Teplow David, B., Neurotoxic protein oligomers--what you see is not always what you get. *Amyloid* **2005,** 12, (2), 88-95. (c) Haass, C.; Selkoe, D. J., Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta -peptide. *Nat. Rev. Mol. Cell Biol.* **2007,** 8, (2), 101-112 (d) Nilsberth, C.; Westlind-Danielsson, A.; Eckman, C. B.; Condron, M. M.; Axelman, K.; Forsell, C.; Stenh, C.; Luthman, J.; Teplow, D. B.; Younkin, S. G.; Naeslund, J.; Lannfelt, L., The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. *Nat. Neurosci.* **2001,** 4, (9), 887-893. (e) Lesne, S.; Koh, M. T.; Kotilinek, L.; Kayed, R.; Glabe, C. G.; Yang, A.; Gallagher, M.; Ashe, K. H., A specific amyloid-beta protein assembly in the brain impairs memory. *Nature (London, U. K.)* **2006,** 440, (7082), 352-357.

[19] Chiti, F.; Dobson, C. M., Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* **2006,** 75, 333-366.

[20] Janek, K.; Rothemund, S.; Gast, K.; Beyermann, M.; Zipper, J.; Fabian, H.; Bienert, M.; Krause, E., Study of the Conformational Transition of Abeta (1-42) Using D-Amino Acid Replacement Analogues., **2001,** 40, (18), 5457-5463.

[22] Engel AK, S. W., Temporal binding and the neural correlates of sensory awareness. *Trends. Cogn. Sci.* **2001**, 16-25.

[23] 1. Ribary, U.; Ioannides, A. A.; Singh, K. D.; Hasson, R.; Bolton, J. P.; Lado, F.; Mogilner, A.; Llinas, R., Magnetic field tomography of coherent thalamocortical 40-Hz oscillations in humans. *Proc Natl Acad Sci U S A* **1991,** 88, (24), 11037-41.

[24] Crowther, D. C.; Kinghorn, K. J.; Miranda, E.; Page, R.; Curry, J. A.; Duthie, F. A. I.; Gubb, D. C.; Lomas, D. A., Intraneuronal Abeta , non-amyloid aggregates and neurodegeneration in a Drosophila model of Alzheimer's disease. *Neuroscience (Oxford, U. K),* **2005,** 132, (1), 123-135.

[25] Nerelius, C.; Sandegren, A.; Sargsyan, H.; Raunak, R.; Leijonmarck, H.; Chatterjee, U.; Fisahn, A.; Imarisio, S.; Lomas, D. A.; Crowther, D. C.; Stromberg, R.; Johansson, J., alpha -Helix targeting reduces amyloid-beta peptide toxicity. *Proc. Natl. Acad. Sci. U. S. A.,* **2009,** 106, (23), 9191-9196, S9191/1-S9191/13.

[26] Chevallet, P.; Garrouste, P.; Malawska, B.; Martinez, J., Facile synthesis of tert-butyl ester of N-protected amino acids with tert-butyl bromide. *Tetrahedron Lett.,* **1993,** 34, (46), 7409-12.

[27] Huang, Y.; Dey, S.; Zhang, X.; Sönnichsen, F. and Garner, P.; *J. Am. Chem. Soc.*, **2004**, *126*, 4626-4640.

[28] Navath, R. S.; Pabbisetty, K. B.; Hu, L., Chemoselective deprotection of N-Boc group in amino acids and peptides by bismuth(III) trichloride. *Tetrahedron Lett.,* **2005,** 47, (3), 389-393.

[29] Sieber, P., Modification of tryptophan residues during acidolysis of 4-methoxy-2,3,6-trimethylbenzenesulfonyl groups. Effects of scavengers. *Tetrahedron Lett,.* **1987,** 28, (15), 1637-40.

[30] Cynthia, G. F. And Gregg, B. F., *Tet. Lett*. **1993**, 34, 6661-6664.

[31] Chhabra, S. R.; Mahajan, A.; Chan, W. C., Homochiral 4-Azalysine Building Blocks: Syntheses and Applications in Solid-Phase Chemistry. *J. Org. Chem.* **2002,** 67, (12), 4017-4029.