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Synthesis of a New Class of Bis(thiourea)hydrazide Pseudopeptides as Potential Inhibitors of β-Sheet Aggregation

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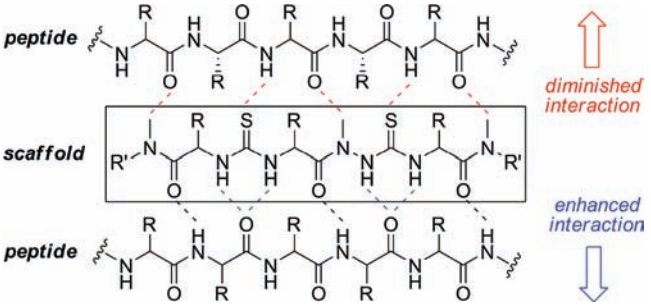
ABSTRACT



The modular synthesis of a novel pseudopeptide scaffold based on a bis(thiourea)hydrazide motif is reported. This compound class is designed to display "amphifinity", i.e. association with a peptide strand on one but not the other face of the scaffold, and hence could potentially inhibit β-sheet aggregation.

The conversion of peptides or proteins from their soluble forms into highly organized fibrillar aggregates is relevant to many neurogenerative diseases, such as Alzheimer's, Parkinson's, Creutzfeldt-Jakob, and Huntington's.[1] These aggregates are generally described as amyloid fibrils or plaques when they accumulate extracellulary. For example in Alzheimer's disease, proteolytic cleavage of the amyloid precursor protein (APP) generates the natively unfolded 39−43 residue amyloid β-peptide (Aβ), which assembles into a characteristic cross-β-structure via the association of β-strands. Finding molecules that prevent Aβ peptide aggregation is the goal for many approaches to develop therapeutics for Alzheimer's and related diseases.[2] There are various approaches to reduce or inhibit Aβ peptide production by the modulation of the proteases, which are involved in the processing of APP,[3] or the development of antibodies,[4] which directly target monomeric Aβ or oligomeric Aβ. In addition, the direct inhibition of oligomerization (Aβ self-assembly) appears to be a promising way to reduce neurotoxicity, mainly since the toxic forms of Aβ are soluble oligomers, as small as dimers and trimers.[5] Therefore, several classes of peptidic and pseudopeptidic aggregation inhibitors have

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been developed.[6] It has been shown that short peptide sequences based on the central hydrophobic core, Aβ (16−20) (KLVFF), act as β-sheet blockers.[7] Other approaches deal with the modification of the amide backbone. For example, peptides with incorporated *N*-methyl amino acids,[8] ester linkages,[9] or proline residues[10] have been designed and show inhibition of β-sheet propagation or even disassembly of preformed fibrils.

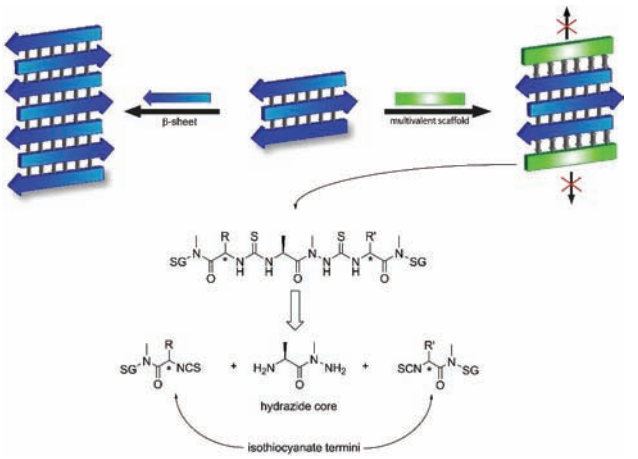


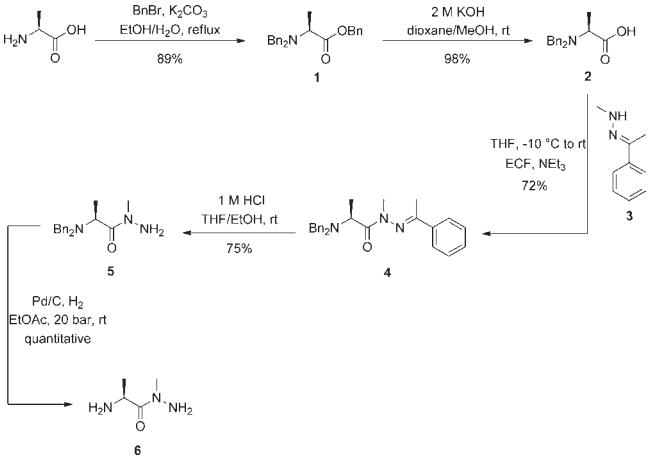
Figure 1. Inhibition of β-sheet aggregation by a multivalent scaffold, its structure, and its retrosynthesis (SG = solubilizing group).

Here, we report a novel scaffold based on bis(thiourea)-hydrazide pseudopeptides (Figure 1), which due to its rational design could potentially inhibit β-sheet aggregation and therefore stabilize a given peptide in its native folded state. Our scaffold is based on several design considerations: On the one hand we take advantage of *N*-methylated amides,[8] thus hydrogen-bonding to one face is blocked and further association of β-sheets as well as homoassociation of the scaffold itself can be prevented. Furthermore, blocking is enhanced by the employment of two thiourea units since the sulfur is a weaker hydrogen-bond acceptor as compared to the amide carbonyl oxygen.[11] On the other hand, hydrogen-bonding to the opposite face is strongly enhanced due to the bidentate binding mode of the more acidic thiourea protons.[11c,12] These *N*-methyl and thiourea motifs are connected via hydrazide linkage to maintain the direction of the peptide backbone, allowing the use of amino acid building blocks for the ease of synthesis and diversification. The resulting target scaffolds should hence present a complementary and a blocking face, which offer the possibility of stronger hydrogen-bonding to the β-sheet on the one face and ensure inhibition of further association on the opposite face, respectively (Figure 1). As our scaffold displays orthogonal binding affinity it can be described as "amphifinic".

To the best of our knowledge, the targeted compound class of bis(thiourea)hydrazides has not been reported thus far. Merely, structure-related peptidyl thiosemicarbazides have been reported recently as intermediates of oxadiazol synthesis.[13] However, these structures lack both multiple thioureahydrazide units as well as *N*-methylation.

A modular synthesis was devised, which in principle allows for the rapid variation of side chain functionality as well as stereochemistry for optimizing the interaction with specific peptide sequences or proteins. A key step in our synthesis is the formation of the two thioureas from an ambident α-*N*-methyl hydrazide and two isothiocyanates, all of which are readily derived from commercially available amino acid building blocks.

Scheme 1. Synthesis of Ambident α-*N*-Methyl Hydrazide Core



The first target for synthesis was the α-*N*-methyl hydrazide core **6** (Scheme 1) from commercially available

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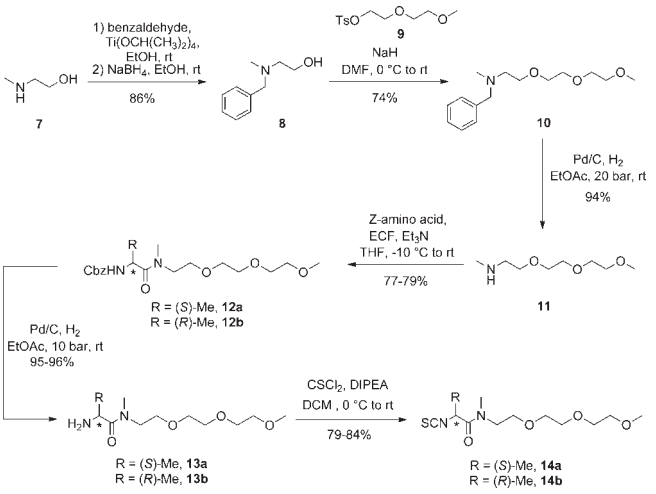
L-Ala-OH as a starting point. Benzylation of L-Ala-OH and subsequent selective deprotection of the C-terminus by saponification provided *N*-protected alanine **2** in very good overall yield (88%). Subsequent regioselective coupling of **2** with hydrazone **3**, masking the primary amino function of *N*-methyl hydrazine by using a ethyl chloroformate protocol[14] furnished **4**. A moderate yield of 72% results from the high instability of the hydrazone functionality. Subsequent hydrazone cleavage followed by quantitative debenzylation by hydrogenolysis provided the α-*N*-methyl hydrazide core **6**.

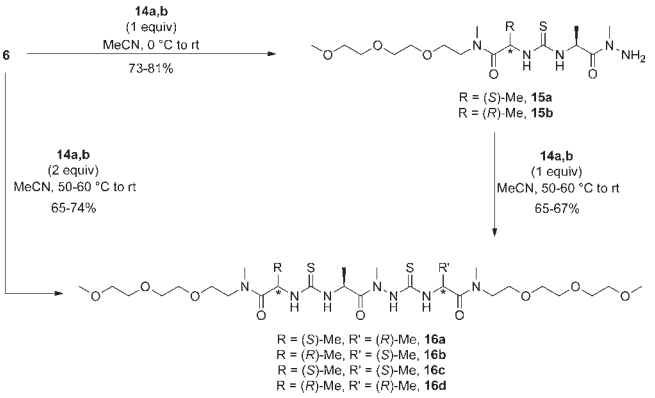
Scheme 2. Synthesis of Isothiocyanate Termini



The first step in the formation of the isothiocyanate terminal building blocks **14a** and **14b** involved the synthesis of the polar triethylenegycol chain **11** (Scheme 2). Benzylation of 2-(methylamino)ethanol via formation of an iminium salt with benzaldehyde and titanium isopropoxide as well as its subsequent reduction with $NaBH_4$ provided **8** (86%). Nucleophilic substitution of **8** with monotosylated diethyleneglycol monomethyl ether **9** gave the benzyl-protected polar triethyleneglycol precursor **10**, which subsequently was deprotected by hydrogenation to obtain the amine **11** in 94% yield. The terminal triethyleneglycol groups of the isothiocyantes were introduced by the reaction of amine **11** with Cbz-protected amino acids (here: L- and D-alanine) using an ethyl chloroformate coupling protocol followed by almost quantitative *N*-deprotection by hydrogenation with Pd/C to obtain both stereoisomers **12a** and **12b**. Finally conversion into the reactive isothiocyanates **14a** and **14b** was carried out in yields of 79−84% using thiophosgene.

Initial coupling of the ambident nucleophile **6** with 1 equiv of isothiocyanates **14a** and **14b**, respectively, yields thioureas **15a** and **15b**, respectively, which can be subsequently coupled with another equivalent of the oppositely configured isothiocyanate (**14a** or **14b**) to obtain the desired target **16a** or **16b**, respectively (Scheme 3). Obviously, the coupling of the two building blocks **6** and **14** can be carried out chemoselectively by carefully controlling the reaction temperature (see Supporting Information). The more reactive primary amine adds rapidly to the isothiocyanate even at low temperatures while the less nucleophilic hydrazide reacts only at elevated temperatures and rather slowly. Consequently, (*R*,*R*,*R*)-thiourea **16c** as well as (*S*,*R*,*S*)-thiourea **16d** can easily be obtained directly by the reaction of nucleophile **6** with 2 equiv of isothiocyanates **14a** and **14b**, respectively, at higher temperatures, i.e. 50−60 °C.

Scheme 3. Chemoselective Synthesis of Bis(thiourea)hydrazides



The synthetic route was demonstrated using alanine-derived building blocks; however, in principle it allows any desired (side-chain protected) amino acid to be employed. Importantly, epimerization can be avoided during synthesis therefore granting access to enantiopure bis(thiourea)hydrazide pseudopeptides with all possible stereochemical variations. Polar triethylene glycol residues were introduced as terminal groups to provide sufficient solubility in aqueous (buffer) solutions, necessary in the context of biological studies. Note that the

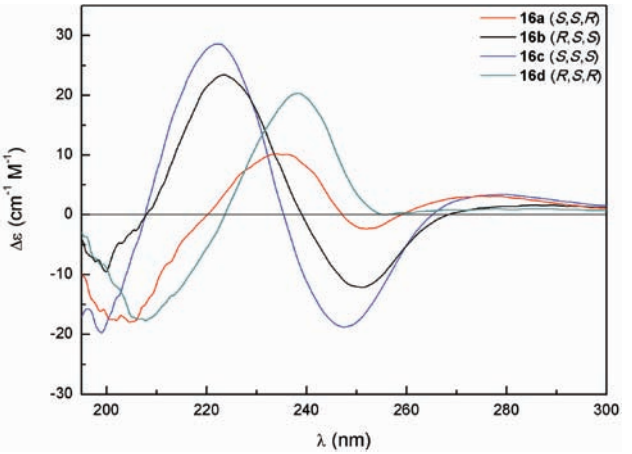


Figure 2. CD spectra of bis(thiourea)hydrazides **16a**−**d** ($c = 4 \times 10^{-4}$ M) in phosphate buffer (10 mM, pH 7.4) at 25 °C.

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terminal residues can be varied freely, thereby offering the possibility for introducing labels or anchors to solid supports, necessary for future automated synthesis. Initial CD spectroscopic studies demonstrate and verify that all bis(thiourea)hydrazides **16a**–**d** display different stereochemistry (Figure 2) as well as the absence of homoassociation (see Supporting Information).

We have described a totally new class of thioureahydrazide pseudopeptides, which due to their "amphifinity" could potentially be able to stabilize peptide sequences, and perhaps even proteins, in their native folded state, thereby preventing misfolding and subsequent aggregation into amyloid-type fibrils. Work, currently ongoing in our laboratories, is concerned with the investigation of the inhibition potential of these scaffolds. Furthermore, detailed conformational analysis of the scaffold and its peptide complexes in solution via NOE-measurements as well as the determination of the association constants for formation of the involved homo- and heteroduplexes will be investigated. In order to explore the full potential of this substance class, a respective solid phase synthesis is being developed to generate libraries for investigating structure–activity relationships to eventually target specific peptides and proteins. This approach should provide a rational means to develop molecules based on our scaffold to sense and potentially cure amyloid and other protein-misfolding related diseases.

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Supporting Information Available. Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.