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Synthesis of (2*R*,3*S*) 3-amino-4-mercapto-2-butanol, a threonine analogue for covalent inhibition of sortases

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Abstract—L-Threonine **2** was converted in seven steps into the protected aminomercaptoalcohol **8**, a threonine mimic. This compound **8** was coupled to various oligopeptides to produce two different tetrapeptide analogues, for example, **11** and **17**, which were shown to inhibit the Sortase enzymes (SrtA and SrtB) via covalent attachment of the thiol groups of **11** and **17** to the catalytically active cysteine residue of the Sortase enzymes.

Many surface proteins on Gram-positive bacteria are covalently anchored to the cell wall by sortase enzymes, a family of novel cysteine transpeptidases.[1] The sortase A (SrtA) protein from *Staphylococcus aureus* is required for bacterial virulence and is the best studied member of this enzyme family. It 'sorts' proteins to the cell wall by processing a conserved C-terminally located LPXTG motif, cleaving the threonine–glycine peptide bond, and attaching the threonine carbonyl to the amine group of lipid II.[2] This lipid–protein intermediate is then incorporated into the peptidoglycan via transglycosylation and transpeptidation reactions of cell wall synthesis. Sortases are an attractive target for new antibacterial agents, since they are widely distributed in a diverse set of pathogens[3] where they are frequently required for virulence.[4] In our ongoing investigations of the structure and function of these enzymes, structures of the apo-SrtA enzyme have been solved,[5] the LPXTG binding site has been coarsely defined,[6] residues critical for catalysis have been identified,[7] and calcium has been shown to activate SrtA by promoting the closure of an active site loop that contacts the substrate.[8] At present, it is not known how these enzymes recognize and process their sorting signals, which can vary dramatically in sequence.[3] For example, although the distantly related sortase B (SrtB) also cleaves the acyl threonine peptide bond, it recognizes a very distinct sorting signal containing the amino acid sequence NPQTN.[4c] To aid in structural studies designed to elucidate the molecular basis of substrate specificity, we have previously prepared several oligopeptides that contain threonine analogues that covalently bind to the thiol of Cys184. These compounds, for example, the vinyl nitrile and vinyl sulfone analogues of threonine, have indeed been useful, but have not yet yielded modified enzymes that are sufficiently homogeneous for detailed structural studies.[6b] Therefore, we have developed a new threonine analogue, which can bind covalently to both SrtA and SrtB by a different mechanism, namely via a disulfide bond. We report the synthesis of (2*R*,3*S*) 3-amino-4-mercapto-2-butanol in the doubly protected form **8** and its incorporation into the two distinct target tetrapeptide sequences for SrtA and SrtB, compounds **11** and **17**, respectively. We also report the binding data for these peptide analogues versus the target enzymes.

Of the many possible protected threonine mercaptomethyl analogues,[9] we chose compound **8** in which the alcohol and thiol functionalities were protected with silyl groups, since the removal of protecting

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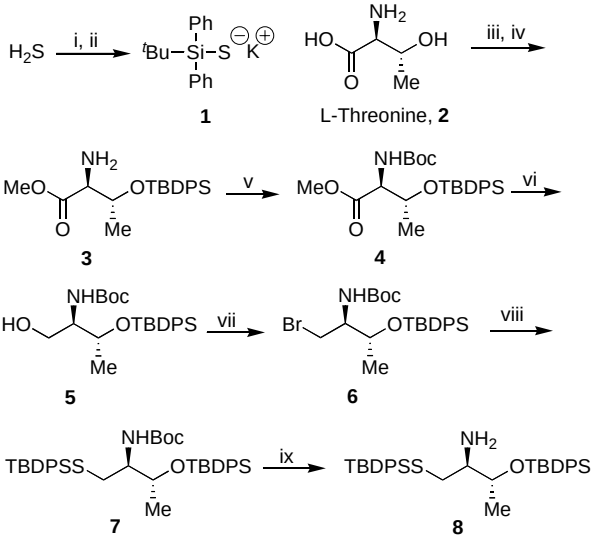
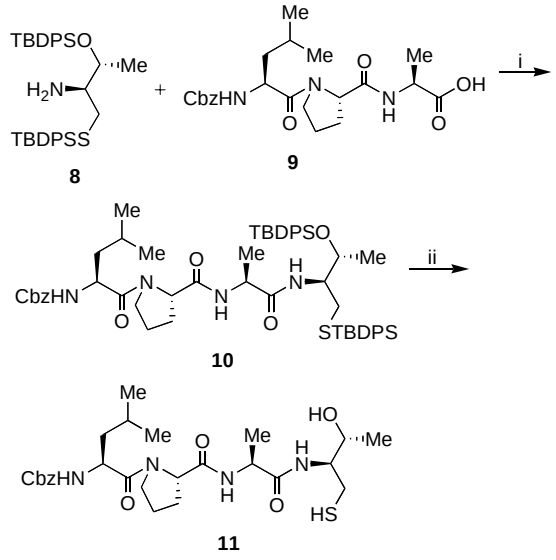
groups should be mild enough as to cause no racemization of any peptide bond. Hydrogen sulfide was converted (Scheme 1) into the *tert*-butyldiphenylsilyl (TBDPS) thiolate **1** in two steps in 72% yield.[10] L-Threonine **2** was converted via standard methods into the silyl-protected methyl ester **3** in an unoptimized yield of 37%. Protection of the amine as the Boc derivative **4** and hydride reduction gave the alcohol **5** in 45% yield. Conversion to the bromide **6** (50%), followed by S_N2 displacement using the thiolate **1**, gave the bis-silylated carbamate **7** in 96% yield. Final Boc removal was quite difficult on account the sensitivity of the TBDPS-protecting groups. Normal acidic conditions removed one or both of the silyl groups in addition to the Boc group. Finally, the following set of conditions were found to work well and reproducibly, namely treatment of **7** with gaseous HCl in anhydrous ethyl acetate at 23 °C for 20 min to afford the desired bis-silylated amine **8** in 82% yield.[11]

With the bis-protected amine in hand, we studied its incorporation into the desired tetrapeptide sequences for SrtA and SrtB. The tripeptide cbz-LPA (leucine-proline-alanine) **9** was prepared in seven steps using standard solution-phase peptide synthesis methodology (Scheme 2). Coupling of **8** with the tripeptide **9** using EDCI and DMAP gave the desired bis-protected SrtA tetrapeptide analogue **10** in good yield. Final deprotection of the two silyl groups with fluoride (TBAF) gave the tetrapeptide analogue **11** in which the carbonyl group of threonine has been replaced with a mercaptomethyl (CH_2SH) unit.

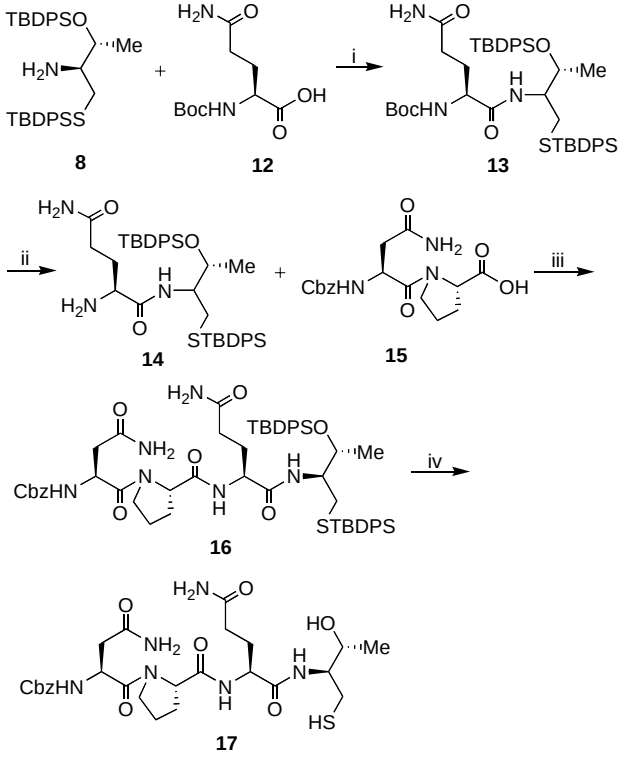


Scheme 1. Reagents and conditions: (i) *n*BuLi, THF, −78 °C → 23°C, 30 min, then 23 °C → −78 °C, TBDPSCI, 30 min, quant.; (ii) KH, pentane, 0 °C, 2 h, 72%; (iii) $SOCl_2$, MeOH, 0 °C → 23 °C, 18 h; (iv) TBDPSCI, imidazole, CH_2Cl_2, 0 °C → 23 °C, 18 h, 37% (two steps); (v) $(Boc)_2O$, Et_3N, MeOH, 18 h, quant.; (vi) LAH, THF, 0 °C, 2 h, 45%; (vii) Et_3N, PPh_3, CBr_4, CH_2Cl_2, 23 °C, 18 h, 50% (viii) **1**, THF, −78 °C, 1 h, then 23 °C, 18 h, 96%; (ix) HCl, anhyd EtOAc, 23 °C, 20 min, 82%.



Scheme 2. Reagents and conditions: (i) EDCI, DMAP, HOBt, CH_2Cl_2, 0 °C → 23°C, 2 h, 86%; (ii) TBAF, THF, 23 °C, 3 h; AcOH, 23 °C, 5 min, 70%.

Due to the somewhat higher reactivity of the amino acid side chains, for example, the amides of the Asn and Gln groups, in the tetrapeptide analogue of the SrtB sequence, a different strategy was used to prepare the SrtB analogue **17** (Scheme 3). Coupling of the bis-silylated amine **8** with α-*N*-Boc glutamine **12** gave the dipeptide analogue **13** in 61% yield. Removal of the Boc group,



Scheme 3. Reagents and conditions: (i) PyBOP, DIEA, CH_2Cl_2, 23 °C, 18 h, 61%; (ii) HCl, anhyd EtOAc, 23 °C, 30 min, 73%; (iii) PyBOP, DIEA, CH_2Cl_2, 23 °C, 1 h, 74%; (iv) TBAF, HOAc, 23 °C, 18 h, 54%.

again using the conditions utilized earlier, for example, HCl in ethyl acetate, gave the amine **14** in 73% yield. The Cbz-protected dipeptide (Asn-Pro) **15** was prepared in four steps using standard solution-phase peptide synthesis methodology. Coupling of the dipeptide analogue **14** to **15** using PyBOP and Hunig's base gave the desired tetrapeptide analogue **16** in 74% yield. Final removal of the two silyl groups with fluoride ion gave the SrtB tetrapeptide analogue **17** in 54% yield. This analogue again has the mercaptomethyl (CH_2SH) group in place of the carbonyl unit of threonine.

To test the viability of these compounds as possible covalent binders, the SrtA tetrapeptide analogue **11** (5 M excess) was added to SrtA (5 μL of 20 μM solution) in a pH 8 buffer (50 mM Tris–HCl, 100 mM NaCl) with and without $CaCl_2$ and incubated at room temperature. Samples were removed periodically and analyzed using reverse-phase HPLC on a C18 column. After 20 h, a large new peak appears, while the original peak for SrtA disappears (Fig. 1). Likewise, when the SrtB tetrapeptide analogue **17** was added to SrtB under similar conditions, a new peak again appears in the reverse-phase HPLC along with the concomitant disappearance of the original peak due to SrtB.

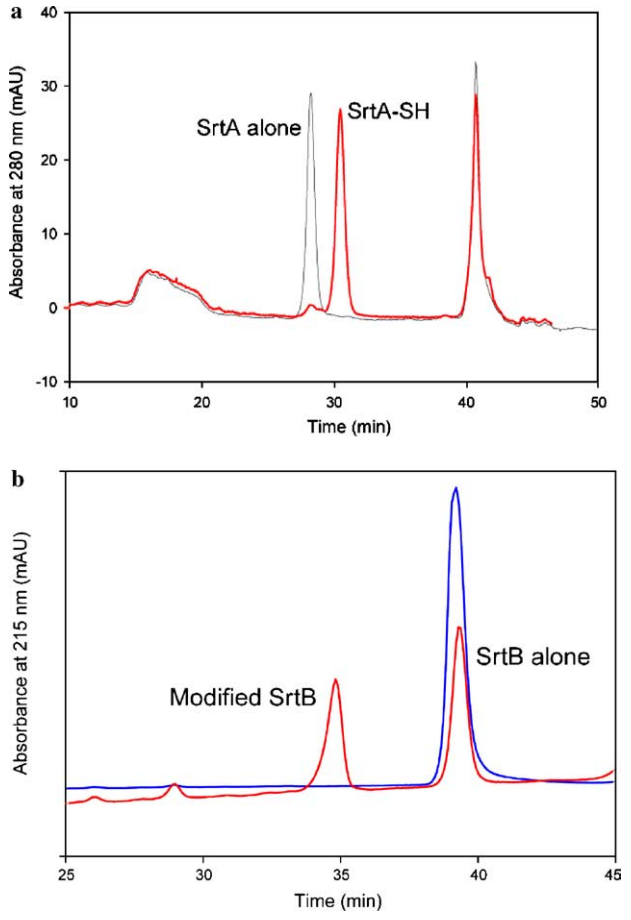


Figure 1. (a) Reverse-phase (C18) HPLC traces of sortase A alone (SrtA alone) and treated with a 10-fold excess of **11** (SrtA-SH). (b) Reverse-phase (C18) HPLC traces of sortase B alone (SrtB alone) and treated with a 5-fold excess of **17** after 12 h at pH 7 (modified SrtB).

In summary, we have developed an efficient method for the synthesis of the bis-protected L-threonine analogue **8** and have used it in the preparation of two tetrapeptide analogues of the sorting sequence for SrtA and SrtB, compounds **11** and **17**, respectively. These compounds covalently modified their respective enzymes at reasonable concentrations. The use of these new covalently bound sortase analogues for the determination of the three-dimensional structure of a bound sortase is currently under study in our laboratories.

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