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Functionalized Analogues of an Unnatural Amino Acid that Mimics a Tripeptide β-Strand

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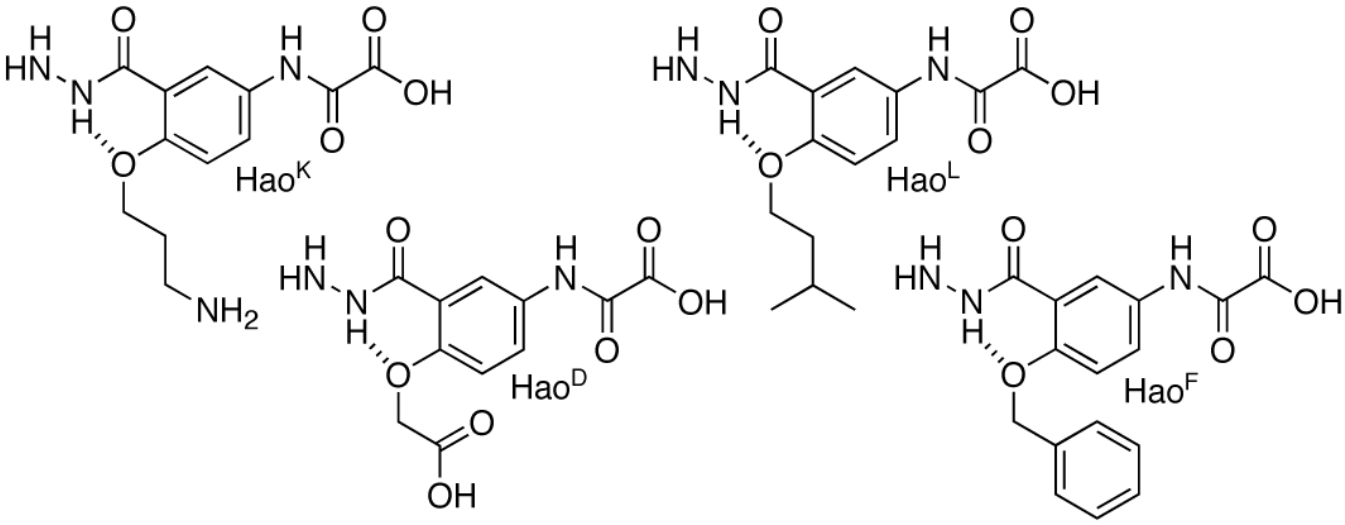
Abstract



This paper introduces polar and hydrophobic variants of the unnatural amino acid Hao, which mimics the hydrogen-bonding functionality of one edge of a β-strand. In these variants, the methyl side chain of Hao is replaced with acidic, basic, and hydrophobic groups. These modifications can impart improved solubility and additional side-chain interactions to peptides containing Hao.

Peptidomimetic templates that mimic or induce helix, turn, or β-sheet structures are useful for studying and controlling the conformations and interactions of peptides and proteins.[1] Our research group previously introduced the unnatural amino acid *Hao* as a tripeptide β-strand mimic that forms hydrogen bonds from only one edge (Figure 1).[2] We have developed Hao-containing peptides that fold to form β-sheet structure, dimerize through edge-to-edge β-sheet interaction, and antagonize β-sheet aggregation.[3] Other research groups have investigated Hao and related structures in peptidomimetic compounds and hydrogen-bonded assemblies.[4,5]

The original unnatural amino acid Hao provides the hydrogen-bonding functionality of the peptide main chain but lacks side-chain functionality. In this paper, we introduce variants of Hao with acidic, basic, and hydrophobic side chains: Hao^K, Hao^D, Hao^F, and Hao^L (Figure 1). We have developed these variants to address specific problems with solubility and folding of Hao-containing peptides that we have encountered in our own research, and we anticipate that these variants and types of side chains will be useful to others.[6,7]

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The amino acid Hao contains a methoxy group that imparts rigidity through intramolecular hydrogen bonding and blocks unwanted intermolecular hydrogen-bonding interactions. To provide additional functionality, we have now replaced the methyl group with aminopropyl, carboxymethyl, benzyl, and isopentyl groups, which respectively resemble the side chains of lysine, aspartic acid, phenylalanine, and leucine. The polar side chains offer the promise of enhanced water solubility and electrostatic interactions, while the hydrophobic side chains offer the possibility of enhanced hydrophobic interactions. To allow use in standard Fmoc-based solid-phase peptide synthesis, we have prepared the Fmoc* derivatives Fmoc*-Hao$^{K(Boc)}$-OH (**1a)**, Fmoc*-Hao$^{D(t\text{-}Bu)}$-OH (**1b**), Fmoc*-Hao^{F}-OH (**1c**), and Fmoc*-Hao^{L}-OH (**1d)** (Figure 2).[8]

The syntheses of Hao analogues **1a**-**1d** are similar to the synthesis of Fmoc*-Hao-OH that we reported previously but require an alkylation reaction to introduce the different side chains and tactical changes to tolerate the functional groups and protecting groups of the side chains. The syntheses of **1a**-**1d** begin with ethyl or allyl 5-nitrosalicylate and involve alkylation of the phenol group to introduce the side chains, conversion of the ester group to the hydrazide, and conversion of the nitro group to the oxamic acid.[9]

Alkylation of ethyl 5-nitrosalicylate with Boc-protected 3-amino-1-bromopropane,[10] *t*-butyl bromoacetate, benzyl bromide, or isopentyl bromide gives ethers **2a**-**2d** (Scheme 1). Alkylation to form **2a** and **2c** proceed smoothly at 70-100 °C. For **2b**, the temperature must be kept below 50 °C to minimize undesired reactions. For **2d**, sodium iodide is added to increase the rate of alkylation. Saponification of the ethyl ester groups of **2a**-**2d** is sluggish at room temperature but occurs in 2-5 h upon heating at reflux in aqueous THF (Scheme 1). Carboxylic acids **3a**, **3c**, and **3d** are readily isolated by neutralization with strongly acidic ion exchange resin (Amberlite IR-120) and removal of THF. Competing reactions during the hydrolysis of **2b** are a problem. To circumvent this problem, we selected the orthogonal allyl protecting group and have used allyl 5-nitrosalicylate to prepare acid **3b**. Alkylation of allyl 5-nitrosalicylate with *t*-butyl bromoacetate to give ether **4** followed by catalytic deprotection with $Pd(PPh_3)_4$ affords **3b** (Scheme 2).

Coupling of acids **3a**-**3d** with Fmoc*-hydrazine[2] gives the Fmoc*-protected hydrazides **5a**-**5d** (Scheme 3). Acids **3a** and **3b** require non-acidic coupling conditions (EDC, HOAt)[11] to avoid loss of the acid-labile protecting groups, while acids **3c** and **3d** can be coupled as the acid chlorides, as in the original synthesis of Fmoc*-Hao-OH.[2] The former conditions are more convenient and higher yielding and should also be suitable for **3c** and **3d** (and Fmoc*-Hao-OH) if desired.

Introduction of the oxamic acid group by reduction of the nitro group, acylation of the resulting aniline group, and hydrolysis of the resulting oxamate ester affords the desired Fmoc*-protected Hao variants (Scheme 4). Reduction of the nitro group of Fmoc*-protected hydrazides **5a**-**5d** gives anilines **6a**-**6d**. Although catalytic hydrogenation of hydrazides **5a**,

[11]Abbreviations:

EDC
N-ethyl-*N*’-(3-dimethylaminopropyl)carbodiimide hydrochloride

HOAt
1-hydroxy-7-azabenzotriazole

DIC
N,*N*-diisopropylcarbodiimide

HCTU
O-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*, *N*-tetramethyluronium hexafluorophosphate (Reference 13).

5b, and **5d** with Pd/C is efficient, it causes the removal of the labile benzyl side chain of hydrazide **5c**. To selectively reduce the nitro group of hydrazide **5c**, we used $SnCl_2$.[12] Acylation of anilines **6a**-**6d** with ethyl oxalyl chloride followed by saponification of the resulting oxamate esters **7a**-**7d** gives Hao analogues **1a**-**1d**. The saponification occurs rapidly, because the oxamate ester groups are especially electrophilic. The resulting oxamate salts are readily converted to the acids with strongly acidic ion exchange resin. Although the oxamic acid group ($RNH\text{-}COCO_2H$) is more acidic than a regular carboxylic acid, the Boc and *t*-butyl ester groups of **1a** and **1b** appear to be stable under typical storage and handling conditions.

The functionalized Hao building blocks **1a**-**1d** are readily prepared in gram quantities and permit the creation of Hao-containing peptides with modified properties. The Fmoc*-Hao^X-OH building blocks **1a**-**1d** can be coupled in solid-phase peptide synthesis using either DIC and HOAt or HCTU and 2,4,6-collidine in DMF as coupling reagents.[11,13-15], We have used building blocks **1a** and **1b** to prepare Hao^K and Hao^D-containing macrocyclic β-sheet peptides **9** and **10** as analogues of macrocyclic β-sheet peptides **8**, which we had reported previously (Figure 3).[3f] Macrocyclic β-sheet peptides **8** form tetramers through β-sheet interactions in water and hold promise as inhibitors of β-amyloid aggregation or as ligands to bind protein β-sheets. Our efforts to study these biologically relevant properties have been hampered by precipitation of peptides **8** with anionic buffers and proteins. Hao^D and Hao^K analogues **9** and **10** have shown improved solubility and have allowed us to proceed with our studies.

These functionalized Hao building blocks increase the arsenal of β-sheet peptidomimetic templates and should be useful tools for studying and controlling the conformations and interactions of peptides and proteins. We will report applications and studies of peptides containing these polar and hydrophobic variants of Hao in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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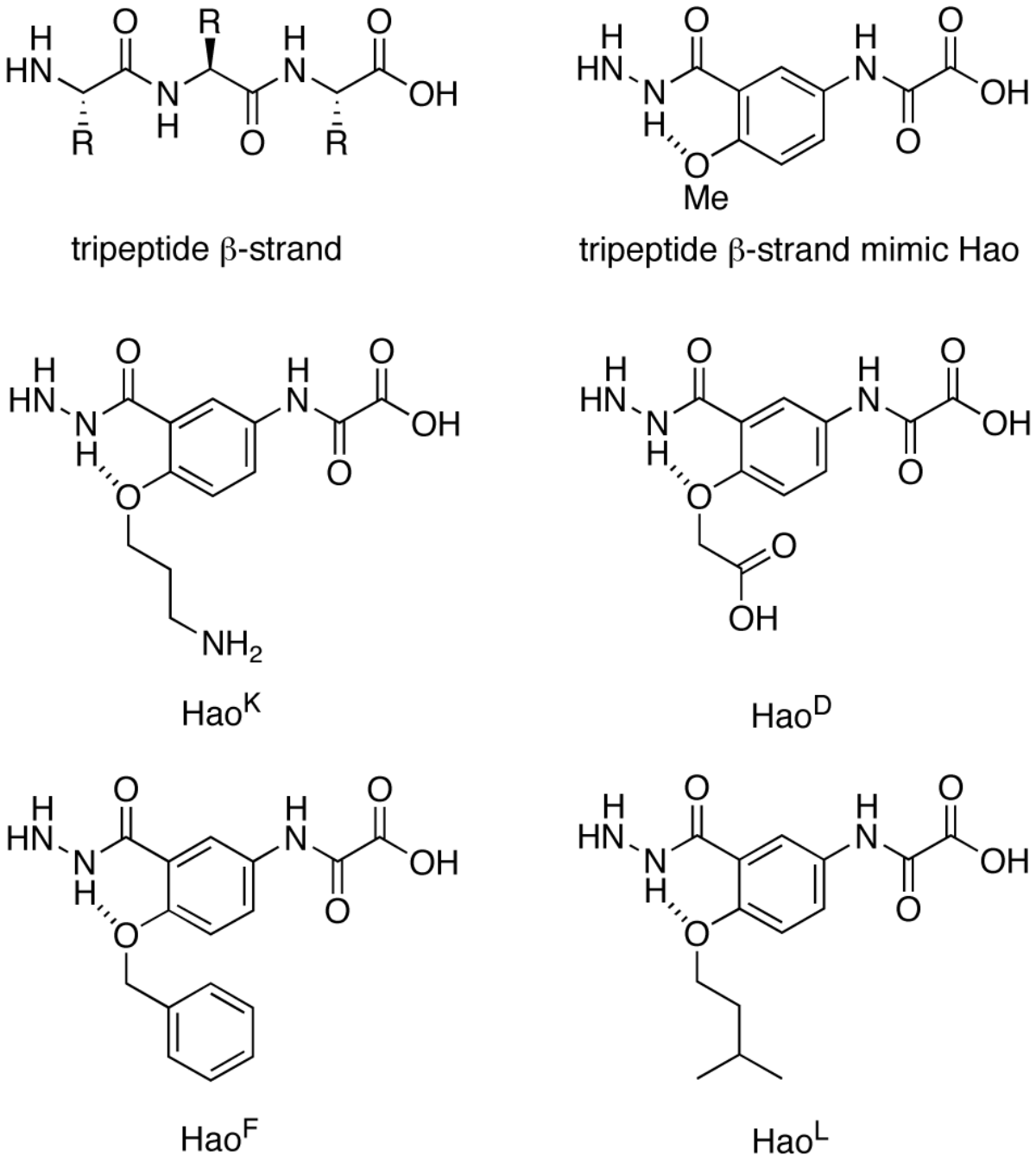


Figure 1.
Tripeptide β-strand, Hao β-strand mimic, and Hao variants.

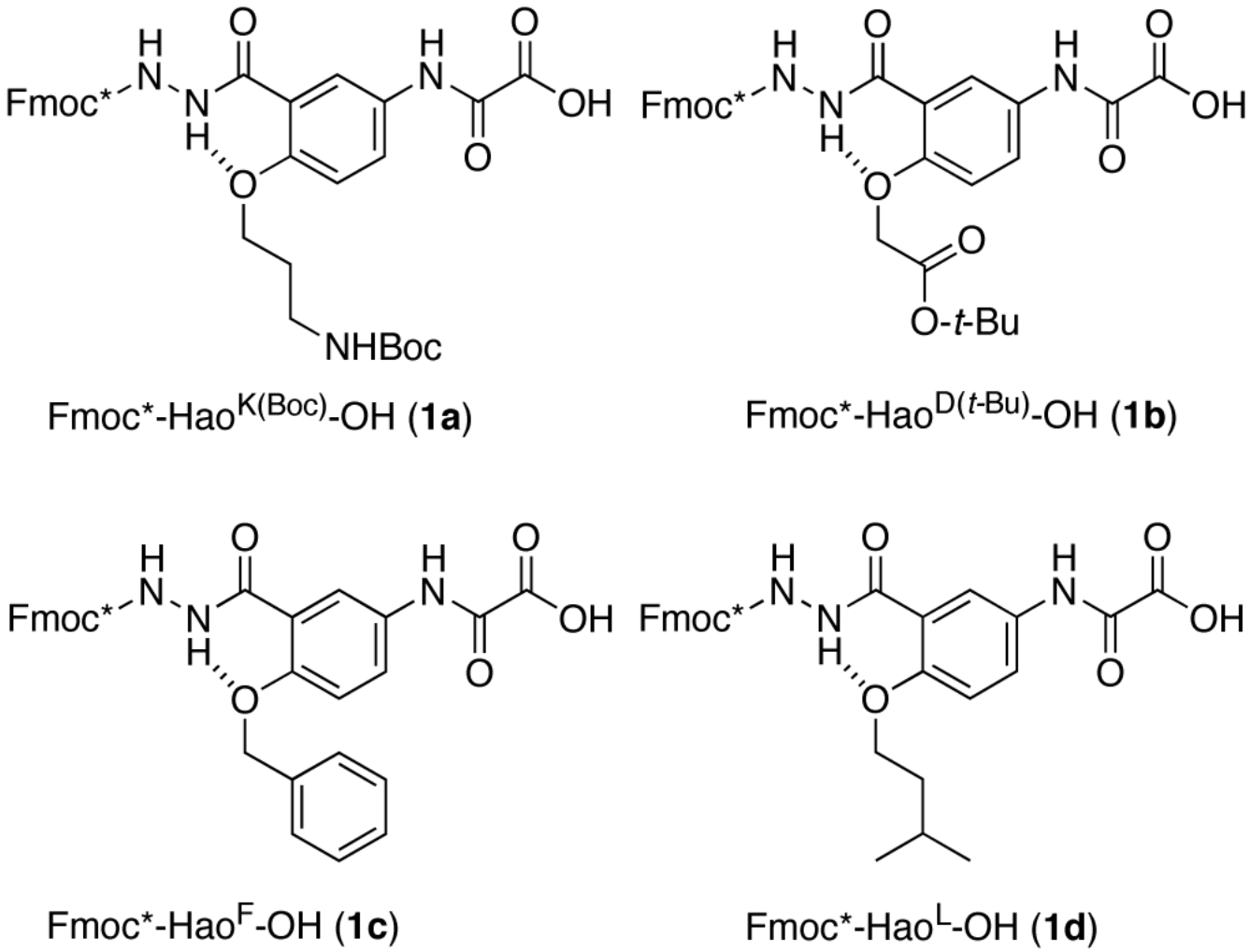


Figure 2.
Fmoc* derivatives of Hao variants.[8]

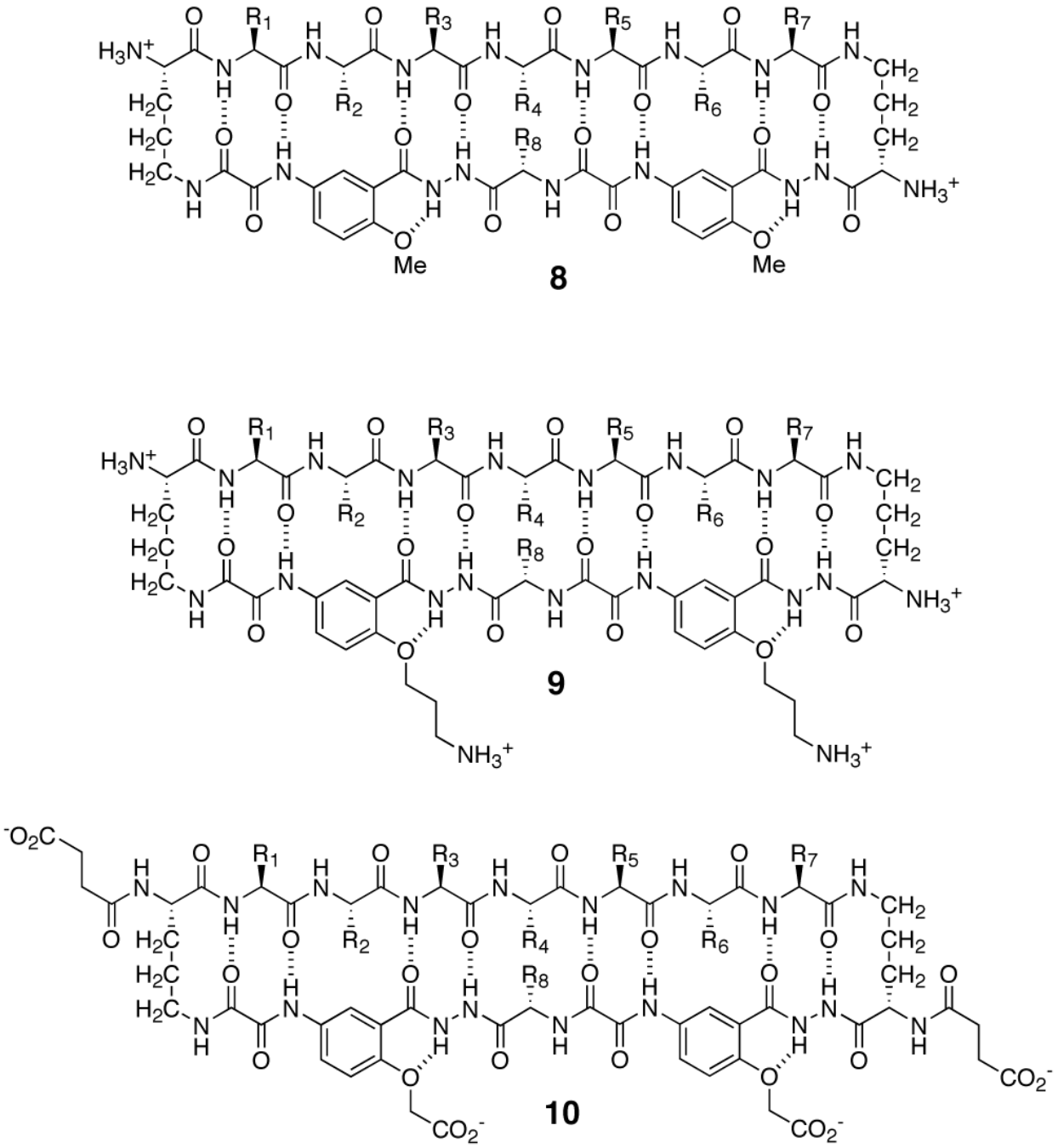
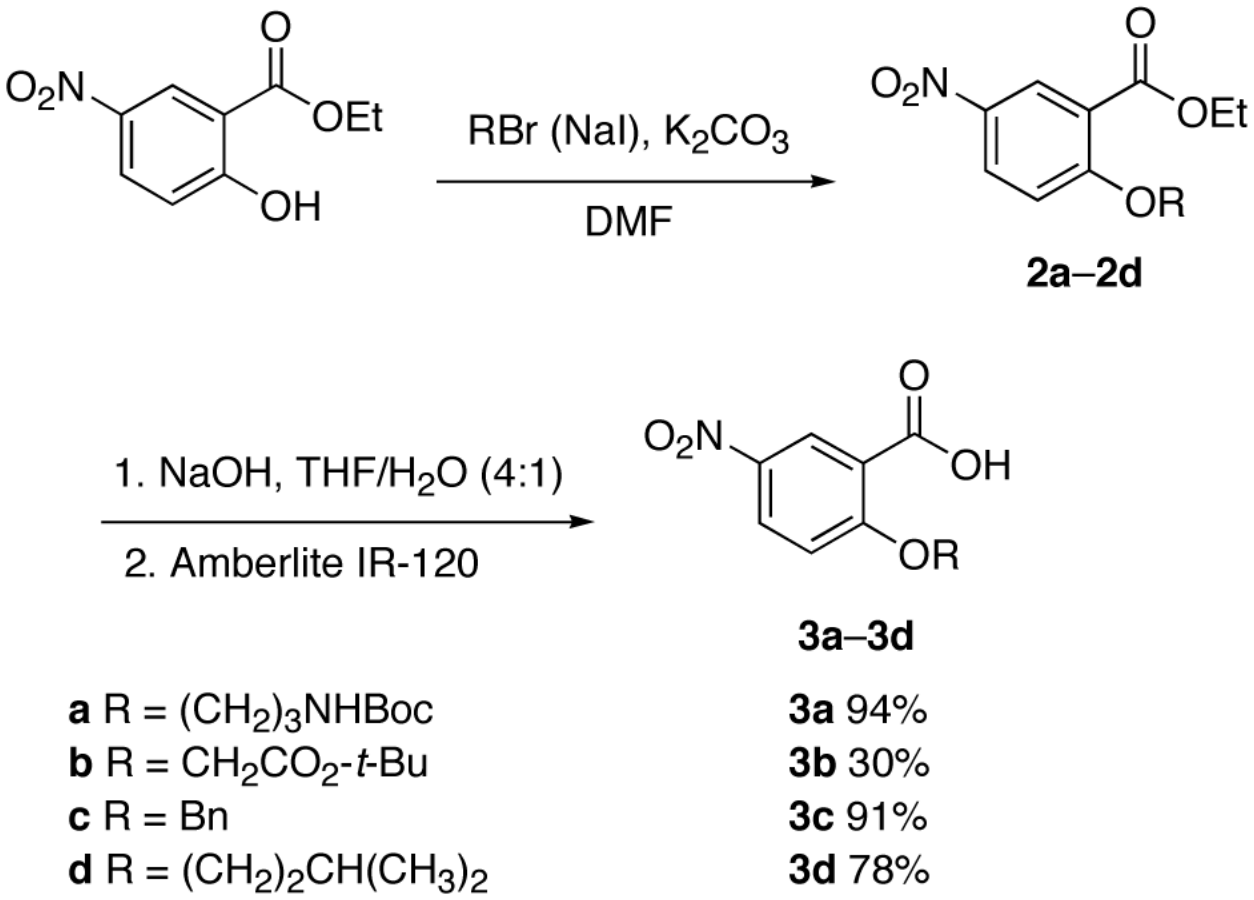
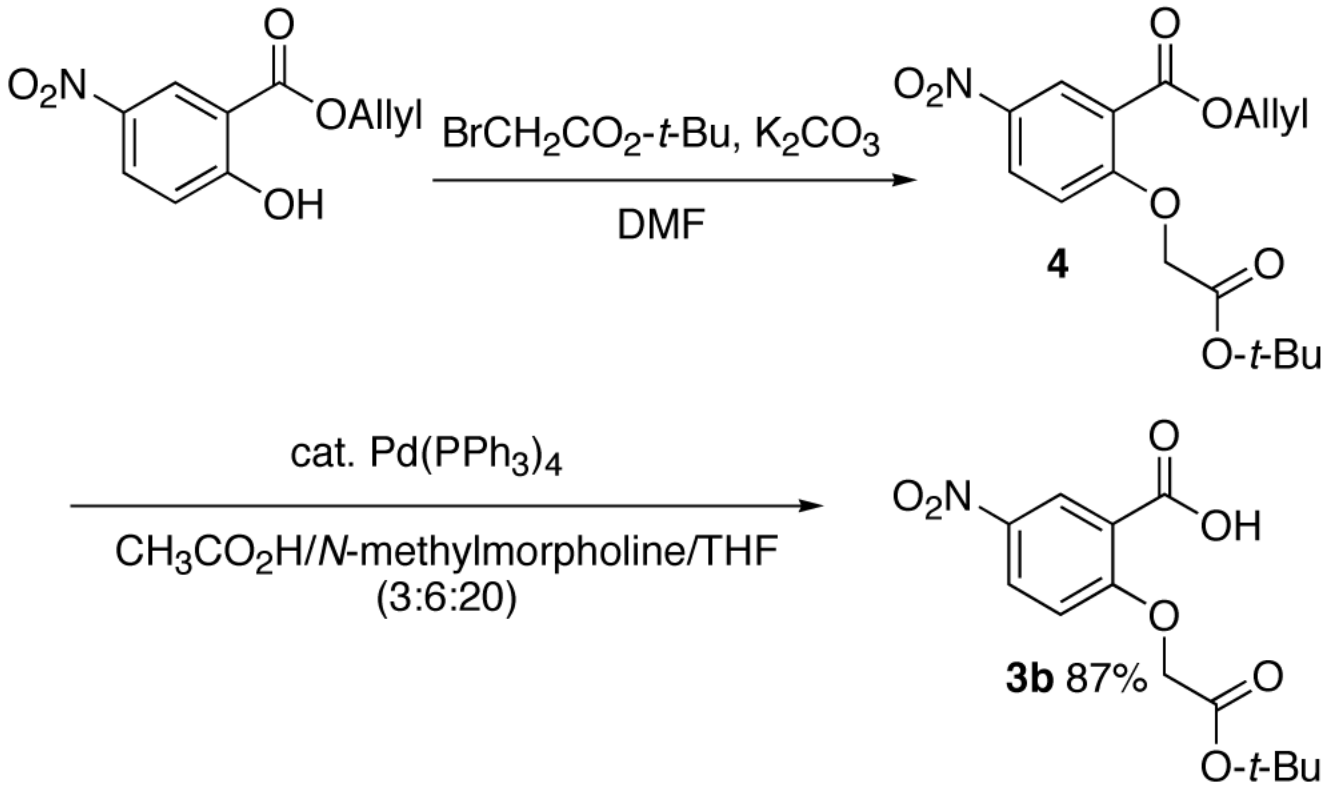


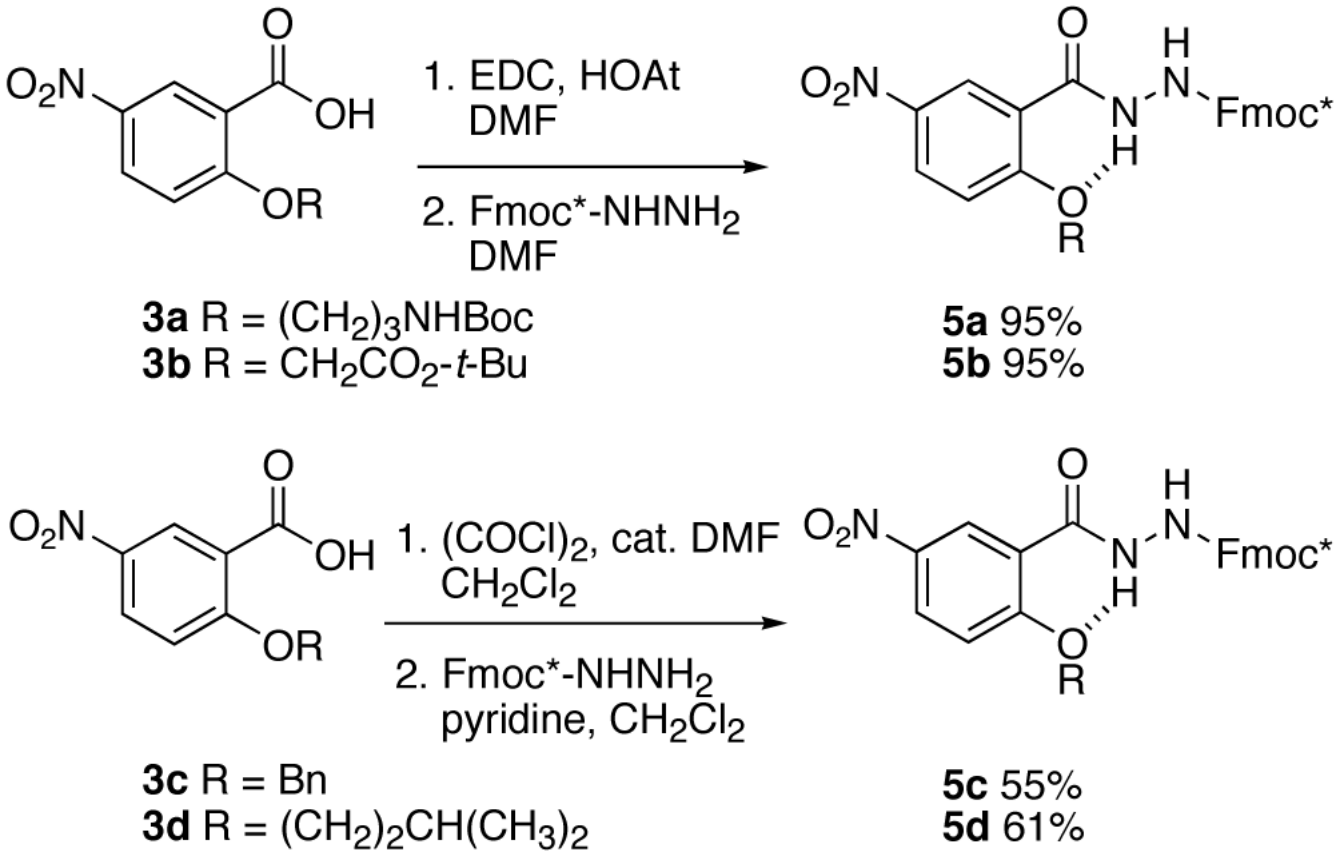
Figure 3.
Hao-, Hao^{K}-, and Hao^{D}-containing macrocyclic β-sheet peptides **8**-**10**.



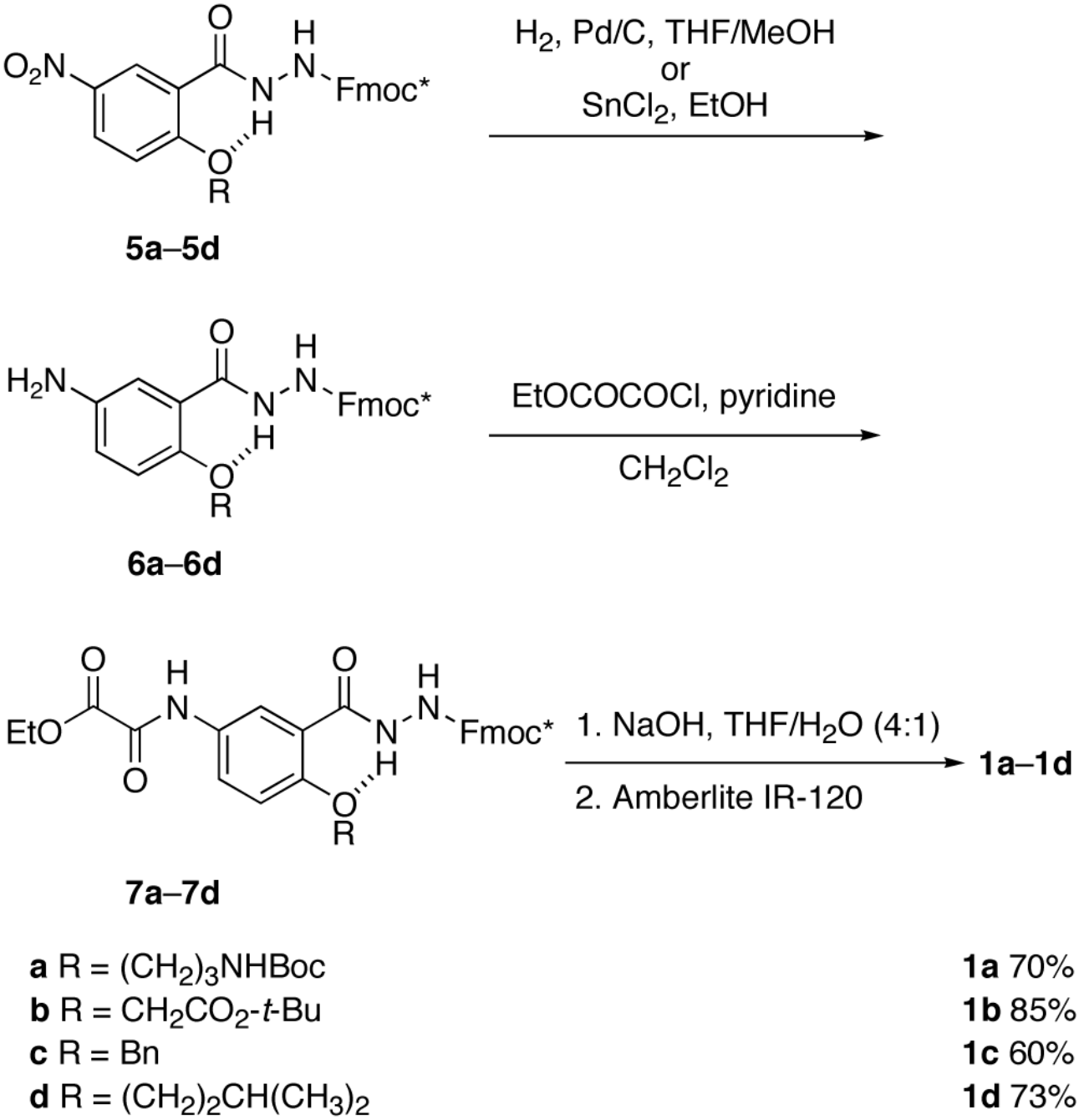
Scheme 1.
Introduction of the side chains.



Scheme 2.
Introduction of the D(*t*-Bu) side chain.



Scheme 3.
Introduction of the Fmoc*-hydrazide group.



Scheme 4.
Introduction of the oxamic acid group.