Efficient Solid-Phase Synthesis of Compounds Containing Phenylalanine and Its Derivatives via Side-Chain Attachment to the Polymer Support

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Combinatorial chemistry, combined with recent advances in robotic screening, has become an important tool in drug discovery.[1] Solid-phase synthesis allows the use of excess reagents to drive the reaction to completion and easy removal of the reagents and side products by simple washing with solvent. Since the introduction of the Merrifield method for peptide synthesis,[2] large libraries of linear biopolymers such as peptides and oligonucleotides as well as organic compounds have been generated.[3] Generally, the synthesis of peptides on a solid support is carried out from the *C*-terminus to the *N*-terminus for a variety of reasons.[4] A more efficient approach to the synthesis of peptides involves the anchoring of the side chain to the polymer, an approach that has been well utilized, especially for head-to-tail cyclization of cyclic peptides. This methodology provides minimal risks for side reactions, such as dimerization and oligomerization, even under reaction conditions of high concentration. Furthermore, this side-chain anchoring strategy allows the generation of more diverse libraries of compounds than the conventional unidirectional way (*C*- to *N*- or *N*- to *C*-elongation). Unfortunately, this useful technology has been applicable only to a few amino acids with polar side chains, such as Asp/Glu (COOH), Cys (SH), Ser/Tyr (OH), Lys (NH_2), and His (imidazole).[5]

Because of the nonpolar nature and steric bulkiness of its side chain, phenylalanine is one of the key pharmacophores in peptidomimetics for favorable hydrophobic interactions with biological targets.[6] Almost every aspartyl protease, such as HIV protease, renin, and cathepsins D and E, has a preference for hydrophobic amino acid side chains at the P_1 position;[7] consequently, peptidomimetics designed to inhibit these enzymes generally contain phenylalanine mimics or other bulky hydrophobic groups at the P_1 position. A large number of naturally occurring linear peptides with antineoplastic activity, such as Dolastatin 10 and 15,[8] and numerous families of cyclic peptides[9] that are hydrophobic also contain at least one phenylalanine residue. Therefore, a new methodology utilizing phenylalanine or masked phenylalanine as the building block would be highly desirable for the rapid preparation of a multitude of compounds with important biological activities.

The most efficient way to design a library of phenylalanine-containing molecules would be to attach the phenylalanine side chain to the polymer support so that the residues at both the *N*- and *C*-termini could be varied, leading to libraries with high diversity. Recently, several novel strategies utilizing resin-bound arylsilane as a "traceless linker" have been developed for solid-phase synthesis of aromatics or heteroaromatic compounds.[10−14] This method allows the attachment of substrates to the support at an inert site within the molecule. Upon cleavage from the resin by protiodesilylation, no trace or memory of attachment to the polymer support is left. Also, silicone-directed *ipso*-substitution of arylsilanes is frequently used for regiospecific introduction of functional groups such as bromine and iodine into the aromatic ring, thereby permitting an even higher degree of diversity in a desired chemical library.[15,16] Libraries of compounds constructed on a solid support using silyl linkages include 1,4-benzodiazepines,[10] biaryls,[12] benzofurans,[12] and tricyclics.[13] We reasoned that an appropriate arylsilyl linkage could be used for tethering phenylalanine to the polymer via its phenyl group, and then further elongation of the amino acid backbone would allow rapid synthesis of phenylalanine-containing molecules. Here we report the design of polymer-bound phenylalanine precursors as new building blocks for solid-phase synthesis and their application in dipeptide synthesis on a solid support.

Scheme 1 illustrates the synthesis of a phenylalanine derivative having a silyl linker attached to the phenyl ring and the loading of this compound to brominated polystyrene resin. A carbon−carbon bond-forming reaction between **2** and the lithitiated bislactim ether[17] **3** in THF at −78 °C provided a mixture of

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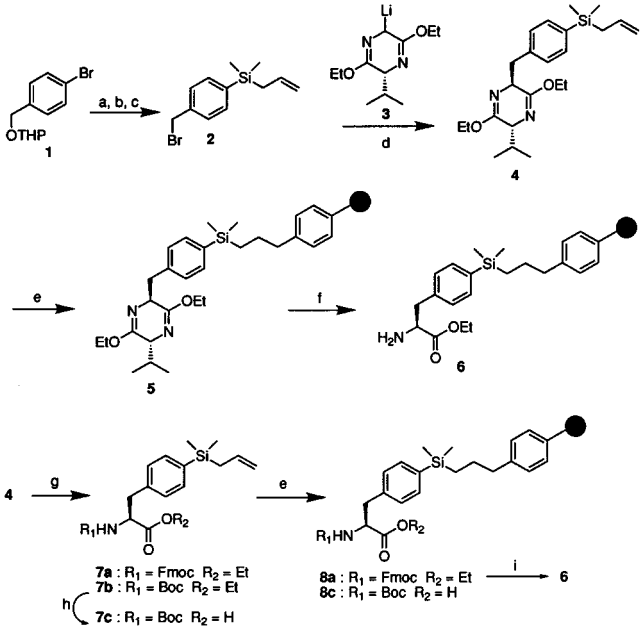
Scheme 1[a]

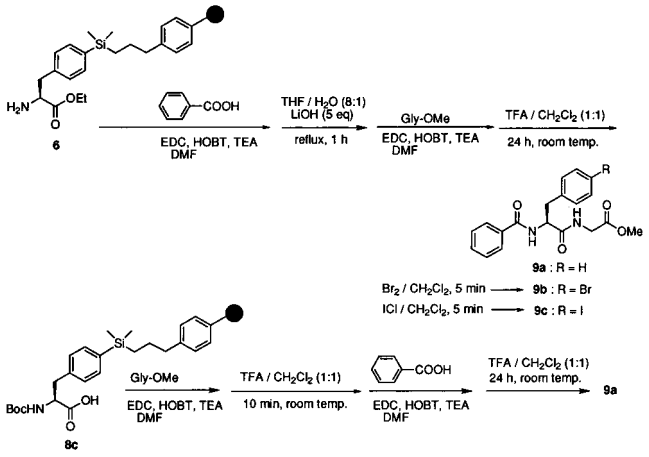


[a] Conditions: (a) *n*-BuLi, THF, −78 °C, then allylchlorodimethylsilane; (b) TsOH (catalytic), MeOH, 16 h; (c) PPh_3, CBr_4, CH_2Cl_2; (d) *n*-BuLi, THF, −78 °C; (e) 9-BBN, THR, 5 h, then bromopolystyrene, DMF, $Pd(PPh_3)_4$, K_2CO_3, 60 °C, 24 h; (f) 8:1 THF/1 N HCl, 3 h; (g) 1:1 MeOH/1 N HCl, 30 min, then FmocCl or $(Boc)_2O$, $NaHCO_3$, dioxane/H_2O; (h) LiOH (5 equiv), 5:1 THF/H_2O; (i) 20% piperidine in DMF.

diastereomers in a ratio of 94:6. The major diastereomer (**4**), isolated in 71% yield after silica gel column chromatography, was attached to the polymer in a two-step sequence. Hydroboration of the allylsilane (9-BBN in THF), followed by in situ Suzuki coupling[18] of the borane complex with bromopolystyrene resin[19] [Pd(0), K_2CO_3, in THF], provided the resin-bound phenylalanine precursor **5**. Treatment of **5** with a solution of THF/1 N HCl (8:1) for 3 h at room temperature afforded polymer-bound phenylalanine ethyl ester **6**, which is ready for further derivatization. Alternatively, **4** can be hydrolyzed under mild acidic conditions (1:1 MeOH/1 N HCl), and the resulting amine can be protected as either an *N*-Fmoc carbamate (FmocCl, $NaHCO_3$) (**7a**) or an *N*-Boc carbamate ($(Boc)_2O$, $NaHCO_3$) (**7b**); saponification of **7b** with LiOH in THF/H_2O gives **7c**. Hydroboration and Suzuki coupling of carbamates **7a** and **7c** with bromopolystyrene resin under the conditions described above provided the resin-bound phenylalanine derivatives **8a** and **8c**, respectively. The loading level of the amino acid derivatives bound to the polymer was determined by the Fmoc release UV/vis assay[20] or by mass balance of the corresponding products released from the resin after treatment of the resin with TFA or Br_2 in dichloromethane.[21]

The utility of the polymer-bound phenylalanine precursors was demonstrated by the preparation of dipeptide analogues from both the *N*- and *C*-termini. As shown in Scheme 2, coupling (EDC, HOBT, TEA in DMF) of **6** with benzoic acid formed the *N*-terminal amide. Hydrolysis of the ester did not proceed at all under the standard conditions (LiOH [5 equiv] in THF/H_2O (8:1), 16 h at room temperature), possibly because of poor swelling properties of the bromopolystyrene resin under the reaction conditions. However, the desired hydrolysis proceeded to completion when the same solution was heated to reflux for 1 h. *C*-Terminal amide coupling was performed on this carboxylic acid with glycine methyl ester under the conditions described above. After completion of the synthesis, cleavage of the desired dipeptide analogue from the resin was carried out with 50% TFA in dichloromethane for 24 h, giving protected dipeptide **9a** in 93% yield. The purity of the crude sample was determined to be about 95% by ^{1}H NMR spectroscopy. Alternatively, cleavage of the dipeptides from the resin by *ipso*-substitution of the silyl group with electrophiles (Br_2 or ICl in dichloromethane for 5 min) provides the corresponding halogen-substituted compounds (**9b**, 97%; **9c**, 94%) with purities higher than 95% as determined by ^{1}H NMR.

Scheme 2



Similarly, but in a reversed reaction sequence, the same compound (**9a**) was synthesized on the solid support starting from the *N*-Boc-protected phenylalanine precursor **8c**. *C*-Terminal amide coupling with glycine methyl ester, followed by deprotection of the Boc group with 50% TFA in dichloromethane for 10 min, and subsequent coupling of the resulting amine with benzoic acid, afforded **9a** in 91% yield after cleavage from the resin (50% TFA in CH_2Cl_2 for 24 h). These two operations demonstrate that small phenylalanine-containing peptides can be prepared on a solid support in any order of reaction sequence desired. The polymer-bound phenylalanine can be modified in either the *N*- or *C*-terminal direction with readily available reagents, such as amines and carboxylic acids, and diversity can be further increased by cleavage of the peptide from the resin with halogens to give *para*-substituted halophenylalanines. These halogenated analogues, then, can be even further elaborated by a variety of alkyl[22] or aryl[18] substitution reactions at the halide.

The arylsilyl linkage was found to be resistant to moderate acidic [1 N HCl/THF (1:8); 50% TFA/CH_2Cl_2 for 10 min] and basic conditions [LiOH, THF/H_2O (8:1), heat] as well as to the general amide coupling reactions. Both *N*-Boc and *N*-Fmoc protection strategies employed for the preparation of dipeptides **9** indicate that this versatility may be of great value for the solid-phase synthesis of complex molecules requiring various orthogonal protection of amine intermediates. Furthermore, this methodology may be applied to cyclic peptide synthesis by head-to-tail cyclization of a peptide on a solid support.

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Supporting Information Available: Complete experimental details for synthesis of the compounds discussed herein (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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