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Synthesis of peptides containing DOPA (3,4-dihydroxyphenylalanine)

Mary J. Sever and Jonathan J. Wilker*

Department of Chemistry, 1393 H.C. Brown Laboratories, Purdue University, West Lafayette, IN 47907-1393, USA

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Abstract—Proteins from coral reefs structures, eggshells, and marine mollusk adhesives all contain the amino acid 3,4-dihydroxyphenylalanine (DOPA). The insoluble nature of these materials has hampered characterization and turned our efforts toward work with small peptide mimics. In this paper, we present the syntheses of various DOPA derivatives: Boc-DOPA, Fmoc-DOPA, DOPA(TBDMS)$_2$, DOPA(TBDPS)$_2$, Boc-DOPA(TBDPS)$_2$, Fmoc-DOPA(TBDMS)$_2$, and Fmoc-DOPA(TBDPS)$_2$ (where Boc=*tert*-butyloxycarbonyl, Fmoc=9-fluorenylmethyloxycarbonyl, TBDMS=*tert*-butyldimethylsilyl, and TBDPS=*tert*-butyldiphenylsilyl). These DOPA compounds were used to prepare peptides of various sequences. The synthetic procedure described provides an efficient route to DOPA-containing peptides in which sidechain deprotection and cleavage from resin can be accomplished in one step.

1. Introduction

The ubiquitous nature of 3,4-dihydroxyphenylalanine (DOPA) touches biological fields ranging from medicine to engineering. Most commonly known for pharmacological value, L-DOPA has been the treatment of choice to alleviate Parkinson's Disease symptoms since the late 1960s.[1] DOPA is also found as an amino acid residue in the proteins of diverse animal species. For the most part, these proteins play structural roles in the synthesis of biological materials. Examples of substances based upon DOPA-containing proteins include coral reef structures,[2] eggshells,[3,4] seashells,[4] and adhesives produced by mollusks.[4–7] In these proteins, DOPA is derived from posttranslational oxidation of tyrosine. Subsequent cross-linking of these soluble proteins creates the requisite, hardened matrices of which these materials are comprised.[3,4] Such cross-linked structures tend to be insoluble and, consequently, difficult to study.

In order to understand the chemistry of these fascinating biological materials, we have turned our attention to work with small, soluble peptides containing the DOPA amino acid residue. Previous reports have shown the successful preparation of peptides containing DOPA.[8–18] In most of these methods, however, protecting groups for the catecholic oxygens of the DOPA sidechain remained intact after standard peptide deprotection and resin cleavage reactions. Additional deprotection steps relying upon rather harsh reagents were required specifically for DOPA.

Our goal was to synthesize peptides containing the DOPA residue employing one step deprotection and cleavage with standard reagents. In this paper, we report the preparation of various DOPA derivatives and their applications to peptide synthesis. The success of our procedure is demonstrated by the preparation of peptides with sequences derived from an adhesive precursor protein from the common blue mussel, *Mytilus edulis*.

2. Results

Solid phase peptide synthesis conditions require protection of both the amine and side chain catecholic oxygens of DOPA.[19] Amine protections are largely reliant upon two groups: *tert*-butyloxycarbonyl (Boc) and 9-fluorenylmethyloxycarbonyl (Fmoc).[19] An equimolar reaction of DOPA and di-*tert*-butyl dicarbonate afforded Boc-DOPA. Likewise, Fmoc-DOPA was prepared by the 1:1 reaction of DOPA and 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl). We chose to focus our synthetic efforts upon Fmoc over Boc chemistry owing to cleaner syntheses and no need to work with gaseous hydrofluoric acid.[20,21]

The final step of Fmoc-based solid phase peptide syntheses involves treatment with trifluoroacetic acid (TFA) to effect deprotection of all residue sidechains and cleavage from the resin. Consequently, we discuss the use of acid labile protecting groups for the DOPA catecholic oxygens. Two acid labile groups employed commonly in catechol

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* Corresponding author. Tel.: +765-496-3382; fax: +765-494-0239; e-mail: wilker@purdue.edu

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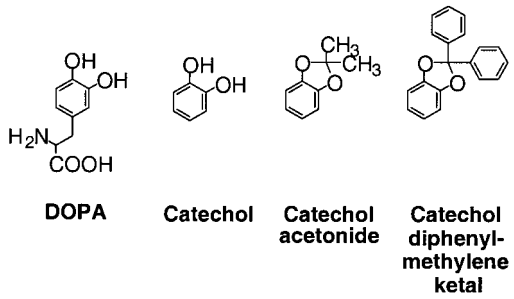


Scheme 1.

protection are the acetonide[22,23] and the diphenylmethylene ketal (Scheme 1).[24–27] Surprisingly, we were unable to prepare acetonide or diphenylmethylene ketal derivatives of DOPA. In our hands, however, comparable protection of catechol worked as presented in the literature.[22,24] We attempted the preparation of DOPA derivatives containing acetonide or diphenylmethylene ketal groups using each of DOPA, Boc-DOPA, and Fmoc-DOPA for starting materials. Reactions of acetone with these DOPA compounds under various conditions of temperature, solvent (toluene, benzene, or THF), and reagents (P_2O_5 or *para*-toluenesulfonic acid) failed to provide the acetonide in acceptable yield. The reagent $H_3C–CBr_2–CH_3$ also did not afford a DOPA-acetonide species. Likewise, protection of DOPA, Boc-DOPA, or Fmoc-DOPA by reaction with $Cl_2C(C_6H_5)_2$ failed to afford the appropriate diphenylmethylene ketal to any appreciable extent.[24–27]

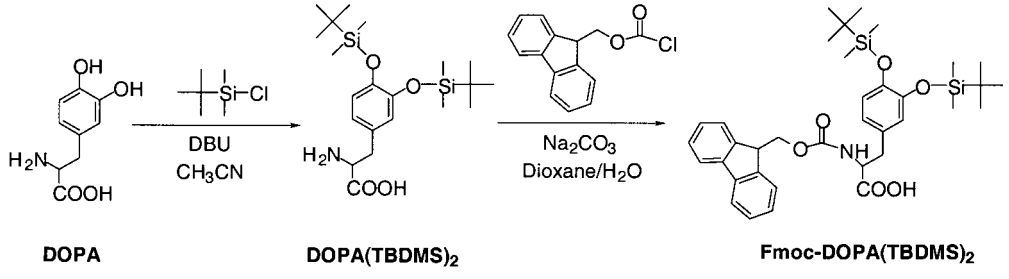
The *tert*-butyldimethylsilyl protecting group, TBDMS, is acid labile[28] and has been installed on DOPA successfully.[29] After preparation of DOPA(TBDMS)$_2$, we effected amine protection in a straightforward manner by reaction with Fmoc-Cl (Scheme 2). Purified Fmoc-DOPA(TBDMS)$_2$ is then suitable for use in solid phase peptide synthesis (vide infra).

The analogous group *tert*-butyldiphenylsilyl, TBDPS, is more stable with regard to hydrolysis relative to TBDMS and preferable for long term storage.[30] Preparation of DOPA(TBDPS)$_2$, however, did not proceed as well as that found for DOPA(TBDMS)$_2$. Reaction of DOPA, TBDPS-Cl, and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) failed to yield DOPA(TBDPS)$_2$ to any appreciable extent. Reaction of TBDPS-Cl with Fmoc-DOPA was also unsuccessful owing to Fmoc deprotection by the DBU used for silylation. Successful installation of TBDPS onto DOPA required using Boc-DOPA for starting material. Intermediate Boc-DOPA(TBDPS)$_2$ was prepared by reaction of Boc-DOPA, TBDPS-Cl, and DBU in acetonitrile. The Boc group was removed by stirring Boc-DOPA(TBDPS)$_2$ for 24 h in ethyl acetate saturated with gaseous HCl. Final amine protection was accomplished by reaction of DOPA(TBDPS)$_2$ with Fmoc-Cl. Scheme 3 summarizes the synthetic route to Fmoc-DOPA(TBDPS)$_2$.

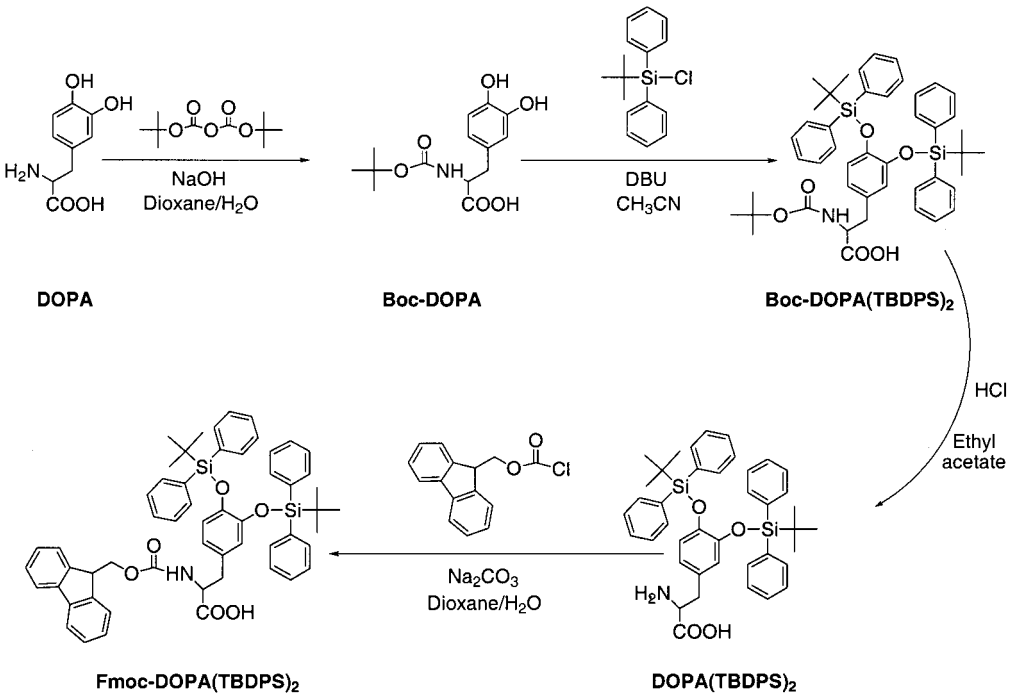
After synthesis of Fmoc-DOPA(TBDMS)$_2$, we wished to ensure that chirality was maintained from the L-DOPA starting material. The Fmoc protecting group was removed by reaction of Fmoc-DOPA(TBDMS)$_2$ with diethylamine to yield DOPA(TBDMS)$_2$. Subsequent action of tetrabutylammonium fluoride (TBAF) provided the desired DOPA. This DOPA was then reacted with *N*-α-(5-fluoro-2,4-dinitrophenyl)-L-valine amide, FDNP-Val-NH_2, a common reagent for chiral resolution by high pressure liquid chromatography, HPLC.[31] FDNP-Val-NH_2 was also reacted with L-DOPA and D,L-DOPA. Fig. 1 shows HPLC traces of each of the three DOPA sources after reaction with FDNP-Val-NH_2. HPLC of the L-DOPA reaction product shows many peaks. The analogous trace from D,L-DOPA shows all the same peaks as L-DOPA along with the addition of four new species observed at 35.03, 45.43, 48.25, and 52.02 min. HPLC analysis of DOPA prepared from Fmoc-DOPA(TBDMS)$_2$ showed only those peaks attributable to L-DOPA. These results indicate that the chirality of Fmoc-DOPA(TBDMS)$_2$ remains intact.

Successful preparations of Fmoc-DOPA(TBDMS)$_2$ and Fmoc-DOPA(TBDPS)$_2$ enabled synthesis of DOPA-containing peptides. Manual, standard solid phase methodologies were used to prepare the peptides.[32] Briefly, amide formation was effected from the amino acid, and 1,3-dicyclohexylcarbodiimide (DCC). In the case of asparagine, 1-hydroxybenzotriazole (HOBT) was used for coupling in addition to DCC. Fmoc removal was accomplished with a 50% solution of piperidine in *N*,*N*-dimethylformamide (DMF). The Kaiser test[33] was used to assess coupling efficiency.

Using Fmoc-DOPA(TBDMS)$_2$, we prepared peptides of sequences Pro-DOPA-Val, Asn-DOPA-Arg-Gly (Scheme 4), and Gly-DOPA-Arg-Gly-Asn-DOPA. Amide formation involving the acids of either DOPA or arginine required excess coupling reagents and reactions times. Excellent coupling efficiencies, however, did result. We chose to cap the N-terminus by acetylation with acetic anhydride. Cleavage from the Rink resin and deprotection of the side chains was accomplished in one step by the use of a solution containing 90% TFA, 5% thioanisol, 3% ethanedithiol, and 2% anisol. In order to optimize time of the deprotection/



Scheme 2.



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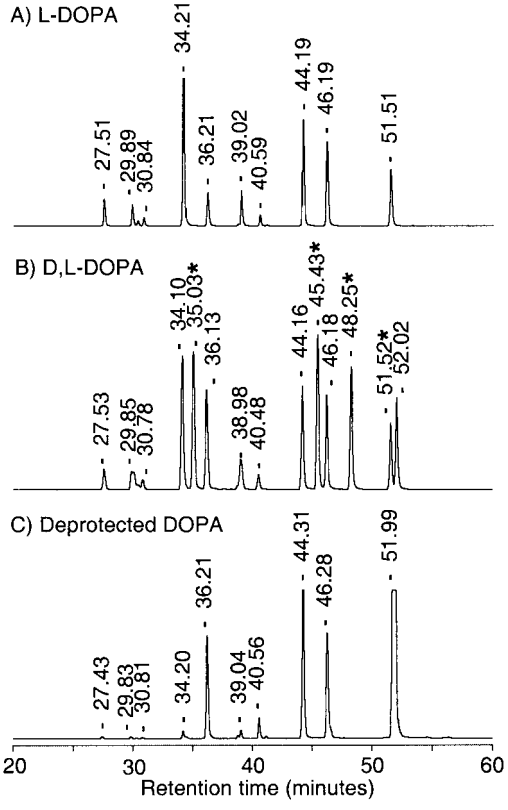
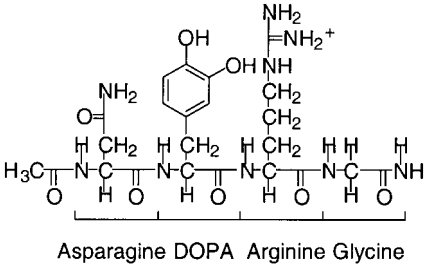


Figure 1. HPLC traces showing absorbance at 340 nm for reaction products of FDNP-Val-NH_2 with A) L-DOPA, B) D,L-DOPA C) fully deprotected Fmoc-DOPA(TBDMS)$_2$. The species attributable to D-DOPA are noted with an asterick.

cleavage reaction, we performed a kinetic experiment using HPLC on the 4-mer peptide, Asn-DOPA-Arg-Gly. As can be seen in Fig. 2, treating the resin with the TFA solution for 4 h resulted in three major peaks. Mass spectral analysis of the isolated peaks provided assignments of the completely deprotected peptide at ~9 min retention time (mass calcd: [M+H]=566.2687. Found: 566.2709) and mono-TBDMS peptides at ~22 and ~26 min (mass calcd: [M+H]=681.4. Found: 681.6). Presumably, these two peaks arise from monosilylation in the 3- and 4-positions. The di-TBDMS peptide remains soluble in the ether precipitating solution according to ultraviolet–visible spectroscopic data (not shown). Reaction of the peptide with TFA solution beyond the initial 4 h showed decreasing amounts of silyl protected peptide and an increased yield of target 4-mer. Peak integration provided the following yields of 4-mer normalized to the 24 h trace: 4 h=2.9%, 8 h=12%, 12 h=42%, and 24 h=84%. Selective nitration[34] of this 4-mer demonstrated the presence of DOPA in the peptide (data not shown). We also prepared the 4-mer Asn-DOPA-Arg-Gly using Fmoc-DOPA(TBDPS)$_2$, but found deprotection/cleavage to be impractical using only this TFA solution for deprotection.

The HPLC traces of Fig. 2 show that the above method provides deprotected peptide after 24 h reaction in the



Scheme 4.

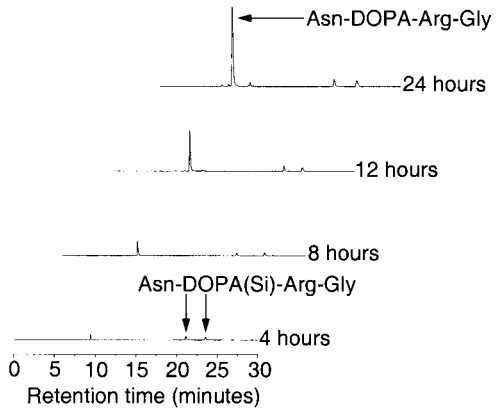


Figure 2. HPLC traces monitored at 280 nm for the deprotection and cleavage reaction of Asn-DOPA-Arg-Gly. Traces shown are for varied reaction times of the resin-bound, protected peptide with a 90% TFA solution. The label Asn-DOPA(Si)-Arg-Gly indicates the mono-TBDMS protected species.

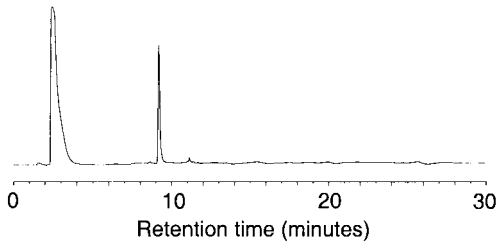


Figure 3. HPLC trace monitored at 214 nm for the peptide Asn-DOPA-Arg-Gly after synthesis and complete deprotection employing fluoride ion. The peptide peak appears at ~9 min. The broad peak at ~3 min is from acetic acid in which the peptide is stored in order to maintain stability of the DOPA residue.

TFA solution. This long TFA treatment, however, is both of a cumbersome length and may not be suitable for use with hydrolytically sensitive peptide sequences. Consequently, we sought deprotection times to minimize exposure to TFA. An excellent means of removing silyl groups is by use of the fluoride anion.[35]

Reactions of TBAF with the silylated 4-mer DOPA peptide, both on the resin and during cleavage reactions, were examined. These studies exhibited a significant dependence upon solvent. Dichloromethane reactions of TBAF and resin-bound peptide always resulted in decomposition of the DOPA peptide after cleavage from resin, as evidenced by absorption at ~380 nm (data not shown). Presumably, this decomposition arises from the basic nature of fluoride[36] and the known sensitivity of DOPA to base.[37] A range of reaction conditions in CH_2Cl_2 were examined, all of which exhibited DOPA decomposition: 1–10 equiv. TBAF per peptide (i.e. 0.5–5 equiv. per silyl group) and reaction times of 1–30 min. Reactions in DMF exhibited slow deprotection and partial decomposition of DOPA. For DMF, conditions were varied amongst 2–5 equiv. TBAF per peptide and times of 1–225 min, none of which were satisfactory. Use of tetrahydrofuran (THF) proved to be the most successful. Reaction conditions examined included 2–50 equiv. of TBAF per peptide and times of 5–150 min. Longer reaction times (>5 min) or greater quantities of TBAF (>6 equiv.) tended toward decomposition of DOPA. We found THF reactions with 5 equiv. of TBAF per peptide for 5 min to work best in terms of product yield and lack of decomposition.

We also examined deprotection of the silylated 4-mer DOPA peptide by reactions with TBAF added to the TFA cleavage/deprotection solution. Again, a range of conditions were studied, including 2–125 equiv. TBAF per peptide and total reaction times of 1–3 h. In the interest of minimizing deprotection times and exposure of the peptide to TFA, we chose 50 equiv. of TBAF per peptide and a time of 1 h. For final optimization of our synthetic method in terms of removing TBDMS groups (determined by HPLC) and total yield of peptide (determined by UV–vis), we combined a TBAF reaction of the resin-bound peptide in THF with addition of TBAF to the TFA cleavage/deprotection solution. In a typical deprotection and cleavage procedure, the resin-bound peptide is reacted with TBAF in THF for 5 min. The resin is then placed in the TFA solution containing TBAF. Peptide is obtained by precipitation in diethyl ether (see Section 4). Analogous reactions with peptide synthesized from Fmoc-DOPA$(TBDPS)_2$ exhibited decomposition (absorption ~380 nm) upon deprotection using these conditions. Fig. 3 shows an HPLC trace of the 4-mer TBDMS peptide deprotected with TBAF according to the above-mentioned procedure. We prepared and deprotected the 3-mer Pro-DOPA-Val and 6-mer Gly-DOPA-Arg-Gly-Asn-DOPA peptides by analogous methods.

3. Discussion

We sought compounds and procedures to enable preparation of DOPA-containing peptides with no necessary deviations from standard protocols. Our chemistry is based upon Fmoc amine protection in order to avoid the use of gaseous HF and obtain cleaner peptide products relative to Boc methods.[20,21] Consequently, we protected the DOPA sidechain catecholic oxygens with acid labile groups. Surprisingly, we could not prepare the acetonide or diphenylmethylene ketal derivatives of DOPA. Analogous reactions with catechol did yield the desired products. Apparently, the organic chemistry of DOPA is distinct enough from catechol to create these differences in reactivity. Addition of two TBDMS protecting groups to DOPA, by contrast, was straightforward and useful in preparation of our final reagent for peptide synthesis, Fmoc-DOPA$(TBDMS)_2$ (Scheme 2). This compound was prepared in high purity and free from common dipeptide side products.[38] Full deprotection of Fmoc-DOPA$(TBDMS)_2$ and reaction with FDNP-Val-NH_2 proved that the chiral integrity of starting L-DOPA persisted throughout synthesis and deprotection.

Application of the TBDPS group to DOPA protection was more complicated than TBDMS. An efficient route to Fmoc-DOPA$(TBDPS)_2$ was found using the intermediates Boc-DOPA$(TBDPS)_2$ and DOPA$(TBDPS)_2$ (Scheme 3). While our work was in progress, a report appeared in which the activated pentafluorophenolate ester of Fmoc-DOPA$(TBDMS)_2$ was prepared.[39] Although this paper did not focus upon synthesis, the authors' use of the TBDMS protecting group is consistent with our findings of compatibility with peptide preparations.

Construction of peptides with the DOPA residue using Fmoc-DOPA(TBDMS)$_2$ has proven to be simple and efficient. Our first synthetic target, Asn-DOPA-Arg-Gly, was based upon sequence data from the adhesive protein Mefp-3 of the common blue mussel *Mytilus edulis* (Scheme 4).[40] In keeping with our goal of developing a synthetic procedure using standard solid phase synthetic methodologies, we found a reaction time of 24 h to be best suited for cleavage from the solid support and sidechain deprotection. Although this procedure worked well, exposure to TFA for such long periods may diminish the utility of this method for peptides of hydrolytically sensitive compositions. Consequently, we explored reactions with fluoride ion to facilitate desilylation of the DOPA residue. Previous reports demonstrated compatibility of fluoride with derivatives of all twenty standard amino acids used in peptide synthesis.[41–43] In our final procedure, we pretreated the peptide with fluoride while remaining bound to the resin. Subsequently, we completed desilylation by addition of fluoride to the TFA cleavage/deprotection solution.

After preparation of Asn-DOPA-Arg-Gly, we examined the generality of this synthetic method for DOPA-containing peptides. The 6-mer Gly-DOPA-Arg-Gly-Asn-DOPA was prepared successfully. Proline and valine are, typically, two of the more difficult amino acids to include in peptides.[21] Thus, we sought a peptide containing proline, valine, and DOPA. Synthesis of the 3-mer Pro-DOPA-Val proceeded smoothly.

In conclusion, we have presented a series of compounds and procedures for incorporating DOPA into peptides. This chemistry requires no significant deviation from standard, solid phase peptide synthesis protocols. Such an easy route to DOPA-containing peptides is sure to facilitate studies of fascinating biological materials such as coral reefs, shells, and molluskan adhesives.

4. Experimental

4.1. General procedures

All syntheses were carried out under an argon atmosphere using standard Schlenk techniques. NMR spectra were recorded on a Varian INOVA 300 spectrometer. Silica gel for column chromatography was 230–400 mesh. TLC plates were stained with aqueous solutions of $Fe(NO_3)_3$ as well as I_2. The following reagents were purchased from Acros Organics: *tert*-butyldimethylsilyl chloride (TBDMS-Cl), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 1,3-dicyclohexylcarbodiimide (DCC), and L-3,4-dihydroxyphenylalanine (L-DOPA). The following were purchased from Advanced Chemtech: acetic anhydride, *N*,*N*-diisopropylethylamine (DIEA), 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl), *N*-α-Fmoc-L-proline (Fmoc-Pro-OH), and *N*-α-Fmoc-L-valine (Fmoc-Val-OH). The following were purchased from Aldrich: tetrabutylammonium fluoride hydrate (TBAF), *tert*-butyldiphenylsilyl chloride (TBDPS-Cl), di-*tert*-butyl dicarbonate, L-3,4-dihydroxyphenylalanine (L-DOPA), D,L-3,4-dihydroxyphenylalanine (D,L-DOPA), and 1-hydroxybenzotriazole hydrate (HOBT). The following were purchased from Novabiochem: *N*-α-Fmoc-L-asparagine (Fmoc-Asn-OH), *N*-α-Fmoc-N^G-2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-L-arginine (Fmoc-Arg(Pbf)-OH), *N*-α-Fmoc-L-glycine (Fmoc-Gly-OH), and Rink Amide resin. Piperidine and diethylamine were purchased from Fisher Scientific.

4.2. High pressure liquid chromatography

Data were obtained using a Waters HPLC system composed of a model 600 controller, a Delta 600E multisolvent delivery system pump, a model 2487 dual wavelength absorbance detector, and a Symmetry C 18 5 μm (3.9×150 mm) column. Optical detection was monitored at 214 and 280 nm. All samples were run for 30 min with gradient proportioning between 0.1% TFA in water and 0.1% TFA in acetonitrile. The mobile phase began at 100% of 0.1% TFA in water and was ramped to 70% of 0.1% TFA in water and 30% of 0.1% TFA in acetonitrile over 15 min. The eluent was continued at this 70/30 mixture for an additional 15 min. Data were collected on a personal computer using ChromPerfect™ software (Justice Innovations).

4.3. Synthesis

4.3.1. *N*-(*tert*-Butyloxycarbonyl)-3,4-dihydroxy-L-phenylalanine (Boc-DOPA). This compound was prepared by modification of a general literature procedure for Boc protection of amines.[32] Dioxane (50 mL), water (25 mL), 1 M NaOH (25.4 mL, 25.4 mmol), and L-DOPA (5.00 g, 25.4 mmol) were stirred together for 10 min. After addition of *tert*-butyl dicarbonate (5.55 g, 25.4 mmol), the reaction was stirred for 20 h. Concentration of the solution in vacuo to approximately 85 mL was followed by cooling on an ice bath. Ethyl acetate (75 mL) was added and the mixture acidified to pH≈2.5 with a dilute solution of $KHSO_4$ (10 g in 100 mL). This mixture was added to a separatory funnel to divide the aqueous and organic layers. The aqueous layer was washed with ethyl acetate (2×30 mL) and all organic fractions were combined. The organic layer was washed with water (3×30 mL), brine (3×30 mL), and dried over anhydrous Na_2SO_4. A brown, foamy solid was obtained after solvent removal in vacuo (6.20 g, 82.0% crude yield).

In a typical purification, crude Boc-DOPA (0.753 g, 2.53 mmol) was loaded onto a silica gel column (2.5×30 cm) and eluted with dichloromethane (500 mL), 1.5% methanol in dichloromethane (4 L), 3.0% methanol in dichloromethane (1 L), and 5.0% methanol in dichloromethane (1 L). Final yield of the colorless solid after purification was 40.3%. ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 9H), 2.55–2.86 (m, 2H), 3.90–4.01 (m, 1H), 6.42–6.96 (m, 4H), 8.57–8.82 (br, 2H). ^{13}C{^{1}H} NMR (DMSO-d_6) δ 28.3, 36.01, 55.6, 78.1, 115.4, 116.6, 119.9, 128.8, 143.8, 144.9, 155.5, 173.9. Mass spec. Calcd for $C_{14}H_{19}NO_6$: [M+H]=298.1291. Found: 298.1299.

4.3.2. *N*-(9-Fluorenylmethyloxycarbonyl)-3,4-dihydroxy-L-phenylalanine (Fmoc-DOPA). Dioxane (36.8 mL), a 10% Na_2CO_3 solution (36.8 mL, 31.8 mmol), and L-DOPA (2.50 g, 12.7 mmol) were stirred on an ice bath 10 min prior to addition of Fmoc-Cl (3.29 g, 12.7 mmol) over 50 min. The reaction was stirred 4 h on an ice bath

and 25 h at room temperature. The cream colored suspension was added to a separatory funnel and washed with ether (3×100 mL). The aqueous layer was cooled on an ice bath and acidified to pH≈3 using 6 M HCl. This aqueous solution was added to a separatory funnel and extracted with ethyl acetate (3×100 mL). Ethyl acetate layers were combined, washed with brine (3×100 mL), and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo to yield 3.12 g, 58.6% of a light brown, foamy solid.

In a typical purification, crude Fmoc-DOPA (0.760 g, 1.81 mmol) was loaded onto a silica gel column (2.5×30 cm) and eluted with dichloromethane (500 mL) and 1.5% methanol in dichloromethane (4 L). Final yield of the colorless solid after purification was 64.6%. ^{1}H NMR (DMSO-d_6) δ 2.59–2.96 (m, 2H), 6.46–6.70 (m, 3H), 7.30 (q, 2H), 7.39 (m, 2H), 7.64 (m, 3H), 7.87 (d, 2H), 8.68 (s, 1H), 8.72 (s, 1H), 12.3–13.0 (br, 2H). ^{13}C{^{1}H} NMR (DMSO-d_6) δ 36.8, 56.7, 66.4, 116.1, 117.2, 120.6, 120.8, 126.0, 126.1, 127.8, 128.4, 129.4, 141.4, 144.5, 144.6, 145.7, 156.7, 174.3. Mass spec. Calcd for $C_{24}H_{21}NO_6$: [M+H]=420.1447. Found: 420.1434.

4.3.3. 3,4-Bis(*tert*-butyldimethylsilyloxy)-L-phenylalanine (DOPA(TBDMS)$_2$). To a stirred solution of TBDMS-Cl (6.25 g, 41.5 mmol) in anhydrous acetonitrile (31.0 mL) was added L-DOPA (2.72 g, 13.8 mmol). The colorless suspension was cooled on an ice bath for 10 min prior to addition of DBU (6.24 mL, 41.4 mmol) over 10 min. The reaction was left stirring on an ice bath for 4 h, followed by further stirring at room temperature for an additional 20 h. Solvent was removed in vacuo and the colorless solid transferred to a fritted glass funnel where it was washed with cold chloroform (150 mL). The solid was dissolved in methanol (150 mL) and filtered. Solvent was removed in vacuo to yield a crude, colorless solid (5.96 g, 101%). Alternatively, addition of cold (−20°C) acetonitrile to the reaction solution results in a colorless precipitate. Filtration and washing with cold acetonitrile yields crude DOPA(TBDMS)$_2$.

In a typical purification, crude DOPA(TBDMS)$_2$ (0.755 g, 1.78 mmol) was loaded onto a silica gel column (2.5 cm×30 cm) and eluted with methanol (1 L). Final yield of the colorless solid after purification was 44.3%. ^{1}H NMR (CD_3OD) δ 0.22 (m, 13H), 1.01 (s, 9H), 1.01 (s, 9H), 2.80–3.28 (m, 2H), 3.70 (m, 1H), 6.74–6.91 (m, 3H). ^{13}C{^{1}H} NMR (CD_3OD) δ −3.7, 19.5, 26.6, 26.7, 37.8, 57.9, 122.6, 123.5, 123.7, 130.8, 141.6, 148.5, 174.0. Mass spec. Calcd for $C_{21}H_{39}NO_4Si_2$: [M+H]=426.2496. Found: 426.2503.

4.3.4. 3,4-Bis(*tert*-butyldimethylsilyloxy)-*N*-(9-fluorenylmethyloxycarbonyl)-L-phenylalanine (Fmoc-DOPA(TBDMS)$_2$). On an ice bath, DOPA(TBDMS)$_2$ (10.8 g, 25.3 mmol), dioxane (150 mL), and a 10% Na_2CO_3 solution (53.8 mL, 63.2 mmol) were combined. The cream colored suspension was stirred 10 min prior to adding Fmoc-Cl (6.55 g, 25.3 mmol). The reaction was stirred 4 h on an ice bath and then at room temperature for 18 h. The suspension was added to a separatory funnel with water (75 mL) and extracted with chloroform (3×75 mL). Chloroform layers were washed with water (3×75 mL) and brine (3×75 mL). After drying over anhydrous Na_2SO_4, solvent was removed in vacuo to yield 13.0 g, 79.2% of light brown foam.

In a typical purification, crude Fmoc-DOPA(TBDMS)$_2$ (6.57 g, 10.1 mmol) was loaded onto a silica gel column (2.5×30 cm) and eluted with dichloromethane (2 L), 0.75% methanol in dichloromethane (2.5 L), 1.5% methanol in dichloromethane (1.5 L), and 3% methanol in dichloromethane (5 L). The resulting colorless solid was obtained in a final, purified yield of 52.0%. ^{1}H NMR (DMSO-d_6) δ 0.14 (s, 6H), 0.16 (s, 6H), 0.94 (s, 9H), 0.95 (s, 9H), 2.66–3.10 (m, 2H), 4.00–4.36 (m, 3H), 6.78 (m, 3H), 7.31 (m, 2H), 7.42 (m, 2H), 7.68 (m, 2H), 7.90 (d, 2H), 12.3–13.1 (br, 1H). ^{13}C{^{1}H} NMR (DMSO-d_6) δ −4.3, 18.0, 25.6, 25.7, 35.8, 46.6, 53.4, 55.7, 65.7, 66.4, 120.0, 120.3, 121.7, 122.3, 125.2, 125.3, 127.0, 127.5, 131.3, 140.7, 143.7, 143.8, 144.6, 145.7, 156.0, 173.5. Mass spec. Calcd for $C_{36}H_{49}NO_6Si_2$: [M+H]=648.3177. Found: 648.3165. Anal. Calcd for $C_{36}H_{49}NO_6Si_2$: C, 66.73; H, 7.62; N, 2.16. Found: C, 66.56; H, 7.68; N, 2.42.

4.3.5. 3,4-Bis(*tert*-butyldiphenylsilyloxy)-*N*-*tert*-butyloxycarbonyl-L-phenylalanine (Boc-DOPA(TBDPS)$_2$). Acetonitrile (7.5 mL), Boc-DOPA (4) (1.01 g, 3.41 mmol), and TBDPS-Cl (2.57 mL, 9.88 mmol) were combined and cooled on an ice bath. After 10 min stirring, DBU (1.54 mL, 10.2 mmol) was added over 15 min. Stirring continued on the ice bath for 4 h and then at room temperature for 40 h. The light brown solution was poured into a separatory funnel with water (50 mL) and chloroform (50 mL). The aqueous layer was washed with chloroform (3×50 mL) and all organic fractions combined. After washing with water (3×50 mL), brine (3×50 mL), and drying over anhydrous Na_2SO_4, the solvent was removed in vacuo to yield 2.93 g, 110.9% of crude, light brown foam.

In a typical purification, crude Boc-DOPA(TBDPS)$_2$ (0.754 g, 9.75 mmol) was loaded onto a silica gel column (2.5×30 cm) and eluted with dichloromethane (500 mL) and 1.5% methanol in dichloromethane (1 L). Final yield of the colorless solid after purification was 43.8%. ^{1}H NMR (DMSO-d_6) δ 0.99 (s, 9H), 1.09 (s, 9H), 1.27 (s, 9H), 2.35–2.46 (m, 2H), 3.61–3.73 (m, 1H), 6.18–6.42 (m, 3H), 7.31–7.53 (m, 12H), 7.62–7.80 (m, 8H). ^{13}C{^{1}H} NMR (DMSO-d_6) δ 18.8, 19.1, 26.3, 26.7, 28.1, 35.6, 54.6, 54.9, 58.0, 77.8, 119.2, 120.9, 121.5, 127.9, 128.0, 130.1, 130.4, 132.2, 132.3, 132.5, 135.0, 135.1, 144.0, 145.1, 152.2, 173.4. Mass spec. Calcd for $C_{46}H_{55}NO_6Si_2$: [M+Na]=796.3466. Found: 796.3488.

4.3.6. 3,4-Bis(*tert*-butyldiphenylsilyloxy)-L-phenylalanine (DOPA(TBDPS)$_2$). Ethyl acetate saturated with HCl was prepared by bubbling HCl gas through ethyl acetate (50 mL) for approximately 10 min. After stirring crude Boc-DOPA(TBDPS)$_2$ (7.95 g, 11.8 mmol) in HCl-saturated ethyl acetate (17.4 mL) for 18 h, the solution was poured into a separatory funnel with water (50 mL). The aqueous later was extracted with ethyl acetate (3×50 mL). Organic layers were combined, washed with water (3×50 mL), brine (3×50 mL), and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo to yield 6.40 g, 80.5% of a colorless solid.

In a typical purification, crude DOPA(TBDPS)$_2$ (1.14 g, 1.69 mmol) was loaded onto a silica gel column (2.5 cm×30 cm) and eluted with chloroform (2 L), 1.0% methanol in chloroform (1.5 L), 3% methanol in chloroform (1 L), and 10% methanol in chloroform (1 L). Final yield of the light brown solid after purification was 46.7%. ^{1}H NMR ($CDCl_3$) δ 1.14 (s, 9H), 1.15, (s, 9H), 2.04 (m, 2H), 6.10–6.41 (m, 3H), 7.14 (m, 4H), 7.26 (m, 2H), 7.30–7.44 (m, 6H), 7.62–7.81 (m, 8H). ^{13}C{^{1}H} NMR ($CDCl_3$) δ 19.6, 26.7, 26.8, 56.3, 76.8, 77.2, 77.4, 77.7, 120.8, 121.0, 121.7, 127.9, 128.0, 129.1, 130.0, 130.2, 132.7, 133.0, 133.1, 135.5, 135.6, 145.4, 146.4. Mass spec. Calcd for $C_{41}H_{47}NO_4Si_2$: [M+H]=674.3122. Found: 674.3136.

4.3.7. 3,4-Bis(*tert*-butlydiphenlysilyloxy)-*N*-(9-fluorenylmethyloxycarbonyl)-L-phenylalanine (Fmoc-DOPA (TBDPS)$_2$). Dioxane (15 mL), a 10% Na_2CO_3 solution (21.6 mL, 18.6 mmol), and DOPA(TBDMS)$_2$ (5.03 g, 7.47 mmol) were combined and stirred on an ice bath for 10 min prior to addition of Fmoc-Cl (1.94 g, 7.49 mmol). The reaction was stirred 4 h on the ice bath and 25 h at room temperature. After pouring the light brown solution into a separatory funnel with water (50 mL) and chloroform (50 mL), the aqueous layer was washed with chloroform (3×50 mL). The combined organic layers were washed with water (3×50 mL) and brine (3×50 mL) before drying over anhydrous Na_2SO_4. Solvent was removed in vacuo to yield 5.54 g, 82.8% of a light brown, flaky solid.

In a typical purification Fmoc-DOPA(TBDPS)$_2$ (4.37 g, 4.88 mmol) was loaded onto a silica gel column (6.5 cm×35 cm) and eluted with dichloromethane (3.5 L) and 0.75% methanol in dichloromethane (8 L). Final yield of the colorless solid after purification was 56.9%. ^{1}H NMR (DMSO-d_6) δ 0.99 (s, 9H), 1.08 (s, 9H), 2.26–2.47 (m, 2H), 3.58–3.74 (m, 1H), 6.19–6.39 (m, 3H), 7.23 (m, 3H), 7.39 (m, 16H), 7.57 (q, 2H), 7.71 (m, 8H), 7.84 (d, 2H), 12.2–12.8 (br, 2H). ^{13}C{^{1}H} NMR (DMSO-d_6) δ 18.8, 19.1, 26.3, 26.6, 35.6, 46.7, 55.1, 65.5, 66.4, 119.4, 120.0, 120.9, 121.5, 125.2, 125.3, 127.0, 127.6, 127.9, 130.1, 130.4, 132.3, 132.4, 132.5, 135.0, 135.2, 140.7, 143.8, 144.2, 145.2, 155.7, 173.2. Mass spec. Calcd for $C_{56}H_{57}NO_6Si_2$: [M+H]=894.3. Found: 894.3. Anal. Calcd for $C_{56}H_{57}NO_6Si_2$: C, 75.05; H, 6.41; N, 1.56. Found: C, 74.68; H, 6.42; N, 1.65.

4.3.8. Deprotection of Fmoc-DOPA(TBDMS)$_2$. Complete deprotection of Fmoc-DOPA(TBDMS)$_2$ was accomplished in two steps. First, the Fmoc amine protecting group was removed with base. In a typical procedure, Fmoc-DOPA(TBDMS)$_2$ (0.250 g, 0.386 mmol), dry acetonitrile (2.5 mL), and diethylamine (28.8 mg, 1.931 mmol) were placed in a 10 mL round bottom flask and stirred for 1 h. During this time, a colorless solid precipitated from solution. The resulting suspension was centrifuged and the supernatant decanted. After several acetonitrile washes, the colorless precipitate was dried under reduced pressure. Second, the TBDMS groups were removed using TBAF in order to bring about complete deprotection. To a 10 mL round bottom flask was added DOPA(TBDMS)$_2$ (90.0 mg, 0.210 mmol), THF (2.0 mL) and TBAF (0.111 g, 0.420 mmol). Within 20 min, a colorless precipitate formed. The suspension was centrifuged and the supernatant removed. The remaining colorless solid was washed three times with THF and dried under reduced pressure.

4.4. Chirality determination

Stock solutions of 200 mM amino acid in 1 M HCl were prepared for L-DOPA, D,L-DOPA, and DOPA deprotected from FmocDOPA(TBDMS)$_2$. A 100 mM solution of FDNP-Val-NH_2 in acetone was also prepared. For derivitization of each amino acid sample, 25 μL of the 200 mM DOPA solution, 25 μL of 1 M $NaHCO_3$, 50 μL of 100 mM FDNP-Val-NH_2 in acetone, and an additional 40 μL of 1 M $NaHCO_3$ were combined and heated for 1 h at 40°C.[31] After heating, 20 μL of 2 M HCl was then added and the samples lyophilized overnight. The resulting solid was dissolved in 1.0 mL of dimethyl sulfoxide (DMSO) and filtered (0.45 μm) for analysis by HPLC. Optical detection was monitored at 280 and 340 nm. Samples were run for 60 min with gradient proportioning between 0.1% TFA in water and 0.1% TFA in acetonitrile. The mobile phase began at 100% of 0.1% TFA in water and was ramped to 60% of 0.1% TFA in water and 40% of 0.1% TFA in acetonitrile over 50 min. The eluent was continued at this 60/40 mixture for an additional 10 min.

4.5. Peptide synthesis

Preparation of the 4-mer Asn-DOPA-Arg-Gly is typical and follows: Rink amide resin (0.109 g, 0.0500 mmol) was placed in a peptide synthesis vessel and washed with CH_2Cl_2 (5 mL, 1 min), DMF (2×5 mL, 1 min each), 50% piperidine in DMF (5 mL, 20 min), DMF (2×5 mL, 1 min each), and CH_2Cl_2 (2×5 mL, 1 min each) by rotation of the flask. At this point, the Kaiser test[33] is performed to identify the presence of free amine available for coupling. The resin is further washed with ethanol (2×5 mL, 3 min each), CH_2Cl_2 (2×5 mL, 1 min each), and DMF (2×5 mL, 1 min each). In a separate, dried 100 mL round bottom flask fitted with a drying tube, a coupling solution is prepared containing Fmoc-Gly-OH (0.0603 g, 0.200 mmol) and DMF (2.17 mL). This mixture is stirred until all solid dissolves, then CH_2Cl_2 (2.17 mL) is added and the solution cooled on an ice bath. DCC (0.0380 g, 0.184 mmol) is added and the solution stirred for a further 30 min on the ice bath. Addition of this coupling solution to the peptide synthesis flask containing the resin is followed by a reaction time of 45 min. A solution of 10% DIEA in CH_2Cl_2 (~3 mL) is added and the flask rotated for an additional 15 min. The resin is then washed with DMF (2×5 mL, 1 min each) and CH_2Cl_2 (2×5 mL, 1 min each). The Kaiser test is performed to identify the extent of coupling. Successive addition of amino acids proceeds in a manner analogous to that provided above for washing followed by coupling of glycine to the resin. Couplings for arginine and DOPA required doubling of reaction time (i.e. 90 min rather than 45 min) and equivalents of the Fmoc-protected amino acid (i.e. 8 equiv. rather than 4). Addition of asparagine required preparation of a slightly modified coupling solution: In a dried 100 mL round bottom flask were combined Fmoc-Asn-OH (0.0716 g, 0.200 mmol), HOBT (0.0278 g, 0.200 mmol), and DMF (2.17 mL). After stirring to dissolve the solids, CH_2Cl_2 (2.17 mL) was added and the solution chilled on an ice bath. DCC (0.0825 g, 0.400 mmol) was added and this mixture poured into the peptide synthesis

flask containing the resin. Coupling time was 2 h. After addition of the final amino acid (Asn), we removed the remaining Fmoc and acetylated the N-terminus by the addition of CH_2Cl_2 (4.4 mL), DIEA (43.6 μL, 0.250 mmol), and acetic anhydride (23.6 μL, 0.250 mmol). The reaction proceeded for 1 h, was followed by a CH_2Cl_2 wash (4×5 mL, 1 min each) and assayed by a Kaiser test.

Deprotection of the sidechains and cleavage from the resin for the 4-mer peptide, Asn-DOPA-Arg-Gly, was accomplished by reaction with a solution of 90% TFA, 5% thioanisol, 3% ethanedithiol, and 2% anisol. Reaction times for deprotection/cleavage are discussed in Section 2. Deprotection using the fluoride ion also proved useful. In a typical deprotection and cleavage procedure, the resin-bound peptide (0.016 mmol) was reacted with TBAF (0.077 mmol) in THF (5.0 mL) for 5 min, followed by washing with THF (3×5.0 mL, 1 min each). After drying under vacuum, the resin was placed in 4.0 mL of 90% TFA, 5% thioanisol, 3% ethanedithiol, 2% anisol, and TBAF (0.77 mmol). The peptide was then obtained by precipitation in diethyl ether. This TFA/peptide solution was added to 50 mL of cold ether and stored at −20°C for 4 h. The reaction mixture was centrifuged and the ether supernatant decanted. The precipitated peptide was further washed with 50 mL of cold ether followed by centrifugation and removal of the supernatant. The solid was dried under vacuum. After dissolution in water or 5% acetic acid (v/v), the peptide was purified by HPLC and analyzed by mass spectrometry. Mass spec. Calcd for $C_{23}H_{36}N_9O_8$: [M+H]=566.2687. Found: 566.2709. Analogous procedures were used to prepare the 3-mer peptide, Pro-DOPA-Val, and 6-mer peptide, Gly-DOPA-Arg-Gly-Asn-DOPA. Mass Spec. 3-mer peptide Calcd for $C_{21}H_{30}N_4O_6$: [M+Na]=457.2063. Found 457.602. Mass Spec. 6-mer peptide Calcd for $C_{34}H_{48}N_{11}O_{12}$: [M+H]=802.3484. Found 802.898.

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