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A Laboratory Preparation of Aspartame Analogs Using Simultaneous Multiple Parallel Synthesis Methodology

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Solid-phase peptide synthesis (SPPS), pioneered by Merrifield in 1963 *(1),* is an essential method for synthesizing peptides in the laboratory. In SPPS the "solid state" is a polymeric resin bead with a functional group to which amino acids are chemically attached one-by-one until the desired peptide sequence is achieved. The entire peptide is cleaved from the resin in the final step. SPPS can be used automatically and enables timely and efficient synthesis of natural as well as unnatural peptides. Furthermore, non-proteinergic amino acids can be incorporated in peptides prepared by SPPS, such as D-amino acids and modified amino acids, together with amino acids that cannot be incorporated in proteins expressed in bacteria. The major advantage of SPPS is that during all stages of the synthesis (except the last step) the product is physically connected to the solid resin, which allows the use of excess reagents, thereby increasing the yield of each step, and facilitates separation from the excess soluble reagents or by-products by filtration or decantation *(2–7).*

Many biological purposes require using numerous peptides that slightly differ in amino acid composition. These groups of similar peptides are called libraries and are subsequently screened for biological activity. The simultaneous multiple peptide synthesis (SMPS) method described by Houghten *(8)* is used to synthesize such libraries on a solid support. This parallel synthesis saves time and reagents. The resin is placed in numbered, perforated polypropylene bags, "tea bags", which are permanently sealed and are ready for the simultaneous solid-phase synthesis of a library of peptides bearing the same sequence apart from single amino acid modifications. Vast arrays of systematically varied structures can be synthesized and evaluated for pharmaceutical, agricultural, and other industrial purposes. A profound advantage of the SMPS method is the concept of parallel and divergent synthesis, demonstrated here by the tea bag methodology.

To synthesize peptides the carboxylic acid group of the amino acid must be coupled to the amino group of the previous amino acid while protecting any functional side chains that are present by using pre-protected residues *(4).* In nature proteins and peptides are synthesized by enzymatically catalyzed dehydration of the carboxy and amino groups from the amino end to the carboxylic end. In SPPS, the synthesis is performed in the opposite direction—from the carboxy to the amino end of the peptide—and the coupling is performed by turning the carboxy group of the protected amino acid into an acyl group. The direction of synthesis reduces racemization. Bistrichloromethyl carbonate (BTC) is a convenient reagent for converting carboxylic acids into acyl chlorides. It is less familiar than the HOBt/HBTU coupling agents[1] used in SPPS but BTC has the following advantages: (i) it mediates difficult couplings of Fluorenylmethoxycarbonyl (Fmoc)-protected amino acids to *N*-alkylated amino acids; (ii) the majority of the couplings proceed quantitatively and without racemization; (iii) low cost; and (iv) reduced reaction time *(9).*

This experiment was performed by final-year undergraduate students, working in pairs, who are familiar with standard methods of purification and characterization in organic chemistry. Basic knowledge in organic chemistry is required. The experiment takes three laboratory periods of six hours each. This experience enriches the students' knowledge in topics including protecting groups *(10)* and coupling agents *(11).* The purification and characterization methods that are proposed in the experimental procedure include high-pressure liquid chromatography (HPLC) *(12),* thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) *(13),* and mass spectroscopy (MS) *(14).* Commercial aspartame and analogs can be used as references.[2]

Less advanced undergraduate students may be able to perform this experiment with an appropriate background, using only HPLC and TLC against reference compounds for molecular identification. Furthermore, we suggest that first- or second-year undergraduate students focus on the synthesis of only one analog and use a coupling agent other than BTC, such as HOBt/HBTU (see the Supplemental Material[W], item 5).

Students synthesize aspartame and analogs in this lab. Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a low-calorie artificial sweetener that was discovered in 1965 by James M. Schlatter, a chemist working for G.D. Searle & Company *(15).* It was approved by the U.S. Food and Drug Administration (FDA) in 1981. Studies conducted with a taste-test panel show that aspartame is 160 times sweeter than sucrose and has a very similar taste to that of sugar. In addition, aspartame can intensify and extend fruit flavors, such as cherry and strawberry, and citrus flavors, such as orange, in foods and beverages.

Experimental Procedure

The peptides were synthesized according to Scheme I on Rink amide MBHA resin using Fmoc chemistry. The resin was placed in three polypropylene bags after a number was marked at the top of each unsealed bag. The bags were closed and the number was permanently sealed into the polypropylene to give an easily readable label. The bags were placed in a polypropylene box into which chemicals were poured directly and removed by decantation. The boxes were shaken with a mechanical shaker. Steps 1, 3, and 5 are Fmoc-deprotection steps in which the reactants were shaken with base (piperidine) followed by

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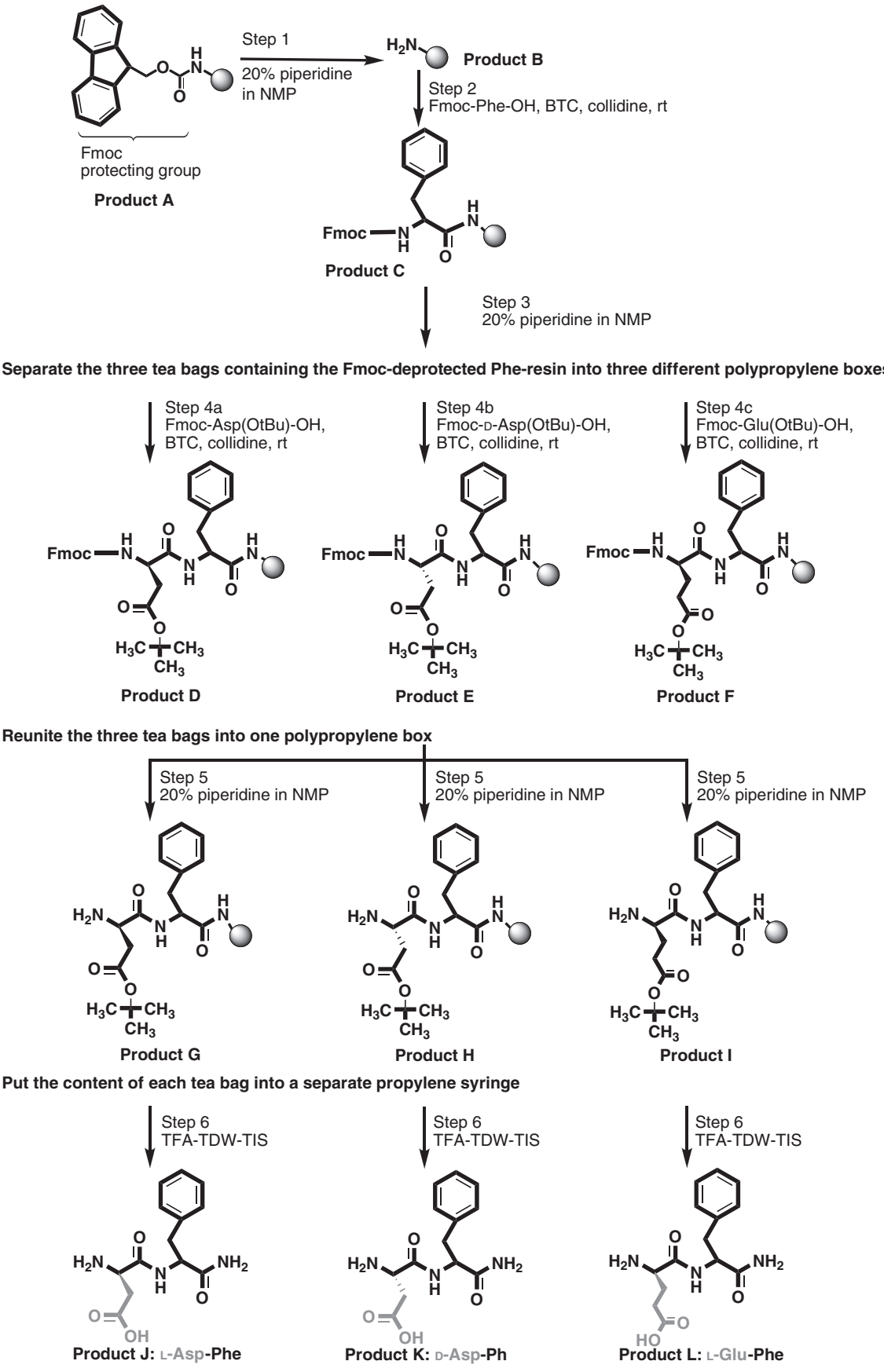

Scheme I. General scheme of the synthesis (circle represents the Rink amide MBHA resin).

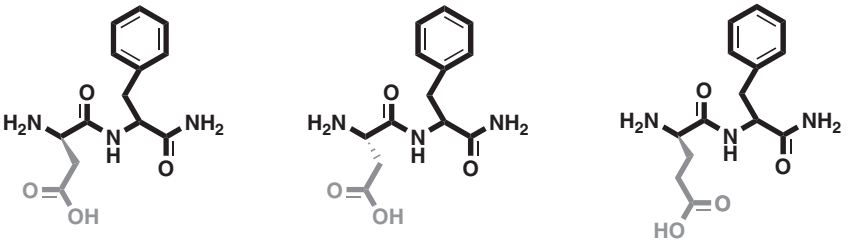

Figure 1. The products of the experiment: H-Asp-Phe-NH_2, H-D-Asp-Phe-NH_2 and H-Glu-Phe-NH_2 (products J, K, and L, respectively).

a wash phase. The steps were performed on all the tea bags simultaneously in the same polypropylene box. All reactions were monitored by the qualitative Kaiser (ninhydrin) test *(16)* to determine completion. In step 2, Fmoc-Phe-OH is coupled to the resin followed by decantation and washing. Step 3 is the second Fmoc-deprotection step, after which each tea bag is placed in a separate propylene box for step 4(a–c): coupling Fmoc-Asp(OtBu)-OH, Fmoc-D-Asp(OtBu)-OH, and Fmoc-Glu(OtBu)-OH, respectively, followed by decantation and washing. All the bags are reunited for step 5, the final Fmoc deprotection, followed by washing and drying. The bags are separated again for step 6, which is acidolytic cleavage and tBu deprotection using trifluoroacetic acid (TFA). After removing the resin by filtration, the cleavage solution was reduced by evaporation under nitrogen to obtain an oily residue of the peptide. Ice-cold ether was then added to dissolve the side product of the tBu-protecting group and to precipitate the peptides, and the side products are then decanted. The products are purified and characterized by HPLC, TLC, MS, and NMR.

Hazards

Students should wear gloves and safety glasses and perform their work inside a fume hood to prevent inhalation of vapors and powders. BTC is very toxic by inhalation and causes burns: it should be weighed in the fume hood. Beware of getting it in eyes, on skin, or clothing. When using this material handle with extreme caution. TFA is extremely irritating to the skin, eyes, and lung tissue; direct participation of the instructor is advisable at the cleavage step. The usual precautions should be observed for working with chlorinated, volatile, flammable, or acidic liquids. None of the reaction procedures involve inherently dangerous operations. We recommend not tasting the products as they may contain dangerous contaminants.

Results and Discussion

The synthesis and characterization of three dipeptide analogs of aspartame (Figure 1) is demonstrated using the tea bag methodology. Solid-phase methodology using the Fmoc-chemistry strategy is employed in this experiment.

General Considerations

All reagents are commercially available and most are common in any organic synthesis laboratory. Protected amino acids, such as Ala, Gly, and Phe, are stable as crystalline solids and are straightforward and safe to use. Residues whose side chains bear functional groups, such as carboxyl (Asp and Glu), hydroxyl or amine, may be used only if proper side chain protection is used. The functional side chains should be orthogonally protected with protecting groups stable to Fmoc/BTC coupling cycles (base) and labile at the final step in which they are removed by TFA cleavage (acid).

Synthesis and Characterization

The syntheses of the three analogs of aspartame, H-Asp-Phe-NH_2, H-D-Asp-Phe-NH_2, and H-Glu-Phe-NH_2, are demonstrated on Rink amide MBHA resin. After swelling the resin (product A, Scheme I), Fmoc deprotection was done: The temporary Fmoc-protecting group, which is stable towards acid but is base labile, was removed using piperidine in NMP (product B, Scheme I) leaving the amine free and rendering the resin reactive. Fmoc-Phe-OH was coupled using BTC-activation (product C, Scheme I) followed by a second Fmoc deprotection. The tea bags were put into three different boxes and three amino acids, Fmoc-Asp(OtBu)-OH, Fmoc-D-Asp(OtBu)-OH, and Fmoc-Glu(OtBu)-OH, were coupled simultaneously in each of the bags (products D, E, and F, respectively, Scheme I), after which the bags were put back into a single box for the last cycle of Fmoc deprotection (products G, H, and I, Scheme I). After separating the bags, the semi-permanent, orthognal side chain protecting group (OtBu) was removed and the peptides were cleaved from the linker of the resin simultaneously in the last step using TFA (products J, K, and L, Scheme I). We used HPLC to purify the dipeptides and TLC, MS, and NMR for their characterization. We found that all the aspartame analogs were not at all sweet, but for safety reasons we do not recommend tasting the student-synthesized products.

Further Investigations

The students can consider possible reasons for the difference in taste between aspartame (H-Asp-Phe-OCH_3) and the closest analog (H-Asp-Phe-NH_2) by drawing both molecules using any molecular drawing software. They can also propose a procedure for synthesizing aspartame itself.

Conclusions

This project was performed by advanced undergraduate chemistry students. The students were required to prepare

the topics addressed in the experiment in advance and write a report including an introduction, results, and discussion. They were mainly assessed by an oral colloquium in which they were required to show their understanding of the chemical mechanisms, the principles of orthogonal protecting groups and SPPS, and how to characterize the compounds by HPLC, MS, and NMR. They were also graded on the product they obtained (degree of purity and yield), their skill in the laboratory (proficiency and efficiency), and their submitted report (theoretical background, analysis of results, methods used and a discussion of the experimental concept). The students had the opportunity to use the modern techniques of SMPS and SPPS, HPLC, MS, and NMR and also successfully reported and completed the experimental task.

Acknowledgment

The authors would like to dedicate this article to the late Murray Goodman, mentor, teacher, and beloved person.

[w]Supplemental Material

Information on chemicals and equipment, reagents, experimental procedures, yields, HPLC and TLC data, NMR spectra, reaction mechanisms, and a list of abbreviations are available in this issue of *JCE Online*.

Notes

1. The abbreviations are defined in the Supplemental Material.[w]
2. For example, aspartame and the analogs can be purchased from Chem-Impex International, Inc: *http://www.chemimpex.com/* Catalog #: 08405 (accessed Sep 2007).

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A Laboratory Preparation of Aspartame Analogues using Simultaneous Multiple Parallel Synthesis (SMPS) Methodology

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Supplemental Material

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Experimental Supplies

Instruments

Sealer – TEW electronic impulse Auto sealer[1] (Figure 1). Shaker[2] – any type will do (Figure 2). NMR data was acquired on a Bruker AMX 300 MHz spectrometer. Chemical shifts are reported in ppm downfield, relative to the internal solvent peaks (10.89 ppm for CF_3COOD). QTof MS spectra were recorded on a QTof II-Micromass apparatus. TLC plates used were Merck silica gel $60F_{254}$ on aluminum. Visualization was achieved either with UV light at 254 nm or by spraying a solution of 0.5% ninhydrin in 1-butanol and heating in an oven at 100ºC for approximately 5 min. The solvent system for TLC is $CHCl_3$:CH_3OH:CH_3COOH (60:30:10). Analytical HPLC was performed on a Merck-Hitachi LaChrom D7000 system (equipped with a L-7400 UV-Vis detector at 220 nm, L-7100 pump and L-7200 auto-sampler). Samples were analyzed on an Atlantis™ RP-18 3 µm, 4.6×150 mm column at 30ºC. The elution program was: Eluents A (0.1% TFA in TDW) and B (0.1 % TFA in ACN) in a linear gradient, starting with 5% B and 95% A with a slope of 2.25 %/min and a flow of 1 ml/min. Retention times refer to the designated system. Preparative HPLC was performed on a Merck-Hitachi 665A system (equipped with a L-4200 UV-VIS detector set at 220 nm, preparative L- 6200A intelligent pump, D – 2500 chromato-integrator). The compounds were purified on an Atlantis™ RP-18 10 µm, 10×150 mm column at 30ºC. Eluents: A (0.1% TFA in TDW) and B (0.1% TFA in ACN) in a linear gradient, starting with 5% B and 95% A with a slope of 2 %/min and a flow of 4.5 ml/min.

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[1] http://www.swery.co.il/ #Other machine (accessed June 2006).

[2] http://www.stjohnassociates.com/products/peptide_wrist-action_flask_shakers.htm (accessed June 2006).

Chemicals	CAS #	Source	Product No.
1-Methyl-2-pyrrolidinone (NMP)	872-50-4		
Acetic anhydride	108-24-7		
Acetonitrile (ACN)	75-05-8		
Acetic acid	64-19-7		
Bis-(trichloromethyl)carbonate	32315-10-9		
Chloroform ($CHCl_3$)	67-66-3		
2,4,6-Collidine	108-75-8		
Dichloromethane (DCM)	75-09-2		
Diethyl ether, anhydrous (Et_2O)	60-29-7		
Diisopropylethylamine (DIEA)	7087-68-5		
Dimethylformamide (DMF)	68-12-2		
Ethanol, 95% (EtOH)	64-17-5		
Fmoc-Asp(OtBu)-OH	71989-14-5	Novabiochem	04-12-1013
Fmoc-D-Asp(OtBu)-OH	112883-39-3	Novabiochem	04-13-1050
Fmoc-Glu(OtBu)-OH	71989-18-9	Novabiochem	04-12-1020
Fmoc-Phe-OH	35661-40-6	Novabiochem	04-12-1030
2-(1H-Benzotriazole-1-yl)-1,1,3,3- tetramethylaminium hexafluorophosphate (HBTU)			
HBTU	94790-37-1	Novabiochem	01-62-0010
N-Hydroxybenzotriazole (HOBt)	123333-53-9	Novabiochem	01-62-0008
Ninhydrin	485-47-2		
Methanol	67-56-1		
Phenol	108-95-2		
Piperidine	110-89-4		
Polypropylene bags (Figure 3)[3]		Scientific Com.	BB2061-125
Polypropylene boxes (Figure 4)[4]		Nalgene	03-311-2C

[3] http://www.scicominc.com/porous_sheets.htm CATALOG #: BB2061 (accessed June 2006).

Polypropylene syringes (Figure 5)[5]		Alltech	210208
Pyridine	110-86-1		
Rink amide MBHA resin		Novabiochem	01-64-0037
Silica gel (TLC sheet)	112926-00-8		
Tri-isopropyl silane (TIS)	6485-79-6		
Trifluoroacetic acid (TFA)	76-05-1		
Trifluoroacetic acid, deuterated	599-00-8		

Hazards

Students should wear gloves and safety glasses and perform their work inside a fume hood to prevent inhalation of vapors and powders. BTC is very toxic by inhalation and causes burns: It should be weighed in the fume hood. Beware of getting it in eyes, on skin or clothing. When using this material handle with extreme caution. TFA is extremely irritating to the skin, eyes and lung tissue; direct participation of the instructor is advisable at the cleavage step. If skin or eyes come in contact with the acid, flush the affected area immediately with water and wash for an additional 15 minutes. The usual precautions should be observed for working with chlorinated, volatile, flammable, or acidic liquids. None of the reaction procedures involve inherently dangerous operations. We recommend not tasting the products as they may contain dangerous contaminants.

Reaction conditions

[4] http://lab-suppliesonline.com/essentials-bottles-square-c-3_173.html (accessed June 2006).

[5] http://www.discoverysciences.com/literature/brochure/464p4.pdf (accessed June 2006).

1. Swelling. In each of the three perforated polypropylene bags (Figure 3) place 0.2 g of dry Rink amide MBHA resin. Mark the bags with pencil and seal them permanently. Place the polypropylene bags on a shaker in a 50 ml polypropylene box (Figure 4). Add 15 ml of NMP and allow swelling for 2 hr[6].
2. Fmoc deprotection. This reaction removes the Fmoc protecting group from the linker amine by shaking with 20% piperidine/NMP (2×15 min). Wash the resin thoroughly (3×NMP) (2×DCM) - to remove the piperidine reagent[7]. The test for free amine using the Kaiser (ninhydrin) test should be positive (Step 1, Scheme 1).
3. Kaiser (ninhydrin) test. To check that the coupling or the deprotection is complete: Ninhydrin reacts with primary amines to give the dye known as Ruheman's purple, a blue color that results, in our case, from the reaction of ninhydrin with free amino termini of the peptidyl resin. The test procedure is as follows:
    a) Open the tea bag and transfer approximately 1-3 mg of the resin to a glass test tube.
    b) Add 3 drops of a solution of 2.5 g ninhydrin in 50 ml ethanol.
    c) Add 3 drops of a solution of 40 g phenol in 10 ml ethanol.
    d) Add 3 drops of a solution of one ml 0.001 M aqueous KCN in 49 ml pyridine.
    e) Mix well and place in a heating block pre-adjusted to 110ºC for 3 min.
    f) Seal the tea bag.

---

[6] Volumes used for swelling and washing are approximately 15 ml – it is important that the tea bags be covered by the solvent.

[7] Each wash was performed by shaking with enough solvent to cover the bags for 2 min, and removing by decantation.

g) Blue colored beads (positive result) indicate: (i) Incomplete coupling of amino acids, or (ii) deprotection of Fmoc protecting groups.

4. Coupling Fmoc-Phe-OH using BTC. Coupling can be performed using BTC or HOBt/HBTU (see item 5 below and Background). Dissolve 0.46 g (1.18 mmol, 3.0 eq) of Fmoc–Phe-OH and 0.12 g (0.39 mmol, 1.0 eq) of BTC in 10 ml of DCM. Cool in the hood in an ice bath. Carefully add 0.44 ml (3.62 mmol, 8.4 eq) of collidine in small portions, shake gently and add to the polypropylene box. Shake 1 min and check pH with a moist pH paper inserted into the vapors of the polypropylene box. If it is not basic (pH 8), add an additional 0.3 ml of collidine. Shake for 30 min and decant[8]. Filter and repeat the last step. Wash the Fmoc-peptidyl resin (5×DCM) (2×NMP). The Kaiser (ninhydrin) test should be negative (Step 2, Scheme 1).

The loading capacity of the resin is used to calculate the amount of amino acids to be used. An example of the weight calculation for the Fmoc-Phe-OH reagent follows.

Reagent	Resin (g)	Loading (mmol g^{-1})	Molar eq	Number of tea bags	MW (g mol^{-1})	**Quantity (g)**
Fmoc-Phe-OH	0.2	0.66	3	3	387.4	**0.46**

$$\text{Fmoc - Phe - OH}\,(g) = \text{Resin}\,(g) \times \text{Loading}\left(\frac{mmol}{g}\right) \times \frac{1mol}{1000mmol} \times \text{Eq} \times \text{Number of bags} \times \text{MW}\left(\frac{g}{mol}\right)$$

[8] Pre-activation of carboxylic acid groups by BTC using collidine as the base is a crucial point and should not be omitted.

5. Coupling Fmoc-Phe-OH using HOBt/HBTU[9]. Coupling can be performed using HOBt/HBTU or BTC (see item 4 above and Background). Dissolve 0.57 g (1.47 mmol, 1.5 eq) of Fmoc–Phe-OH, DIEA 0.07 ml (1.47 mmol, 1.5 eq) and 0.15 g (1.47 mmol, 1.5 eq) of HOBt in 7.5 ml of NMP. Cool in the fume hood in an ice bath. Add 0.19 g (1.47 mmol, 1.5 eq) of HBTU shake gently for 10 min until dissolved, add to the polypropylene box and add 2.5 ml of DCM. Check pH with a moist pH paper inserted into the vapors of the polypropylene box. If not basic (pH 8), add 0.3 ml of DIEA. Shake for 30 min and decant[10]. Filter and repeat the last step. Wash the Fmoc-peptidyl resin (5×DCM) (2×NMP). The Kaiser (ninhydrin) test should be negative (Step 2, Scheme 1).
6. Capping free amine sites on the resin. Prepare a capping solution: 0.93 ml acetic anhydride, 1.72 ml DIEA, 12.5 ml NMP. Add the capping solution to the polypropylene box and shake for 30 min. Filter and repeat the last step. Wash the resin (5×NMP) (2×DCM) (1×NMP). The Kaiser (ninhydrin) test should be negative.
7. Fmoc deprotection. Remove the Fmoc protecting group with 20% piperidine/NMP (2×15 min). Wash the resin (3×NMP) (2×DCM). Monitor the deprotection by Kaiser (ninhydrin) test, which should be positive (Step 3, Scheme 1). Separate the tea bags into three different 50 ml polypropylene boxes marked respectively for each coupling reaction 8-10.
8. Coupling Fmoc-Asp(OtBu)-OH. Dissolve 0.41 g (0.99 mmol, 3.0 eq) of Fmoc–Asp(OtBu)-OH and 0.1 g (0.3 mmol, 1 eq) of BTC in 10 ml of DCM. Cool in an ice bath. Carefully add 0.37 ml (3.02 mmol, 8.4 eq) of collidine in small portions, shake gently and add to the polypropylene box. Shake 1 min and check pH with a moist pH paper inserted into the

[9] In order to avoid using the more common, but toxic, reagent BTC, the amino acids can be coupled using HOBt /HBTU as demonstrated here. This is instead of step 4.

[10] Pre-activation of carboxylic acid groups is a crucial point and should not be omitted.

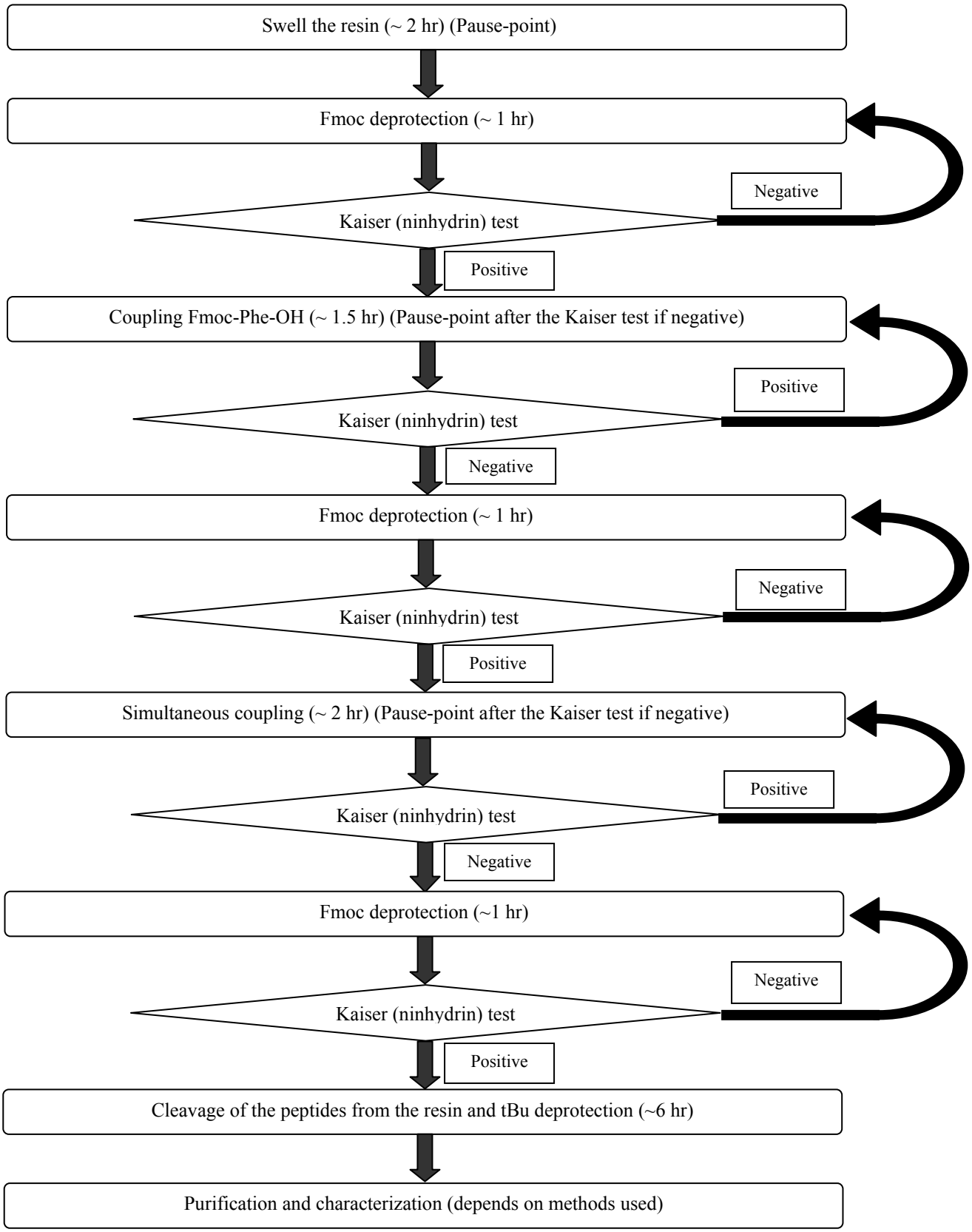
vapors of the polypropylene box. If not basic (pH 8), add 0.1 ml of collidine. Shake for 30 min and decant. Wash the Fmoc-peptidyl resin (5×DCM) (2×NMP). The Kaiser (ninhydrin) test should be negative (Step 4a, Scheme 1).

9. Coupling Fmoc-D-Asp(OtBu)-OH. Dissolve 0.41 g (0.99 mmol, 3.0 eq) of Fmoc–D-Asp(OtBu)-OH and 0.1 g (0.3 mmol, 1 eq) of BTC in 10 ml of DCM. Cool in an ice bath. Carefully add 0.37 ml (3.02 mmol, 8.4 eq) of collidine in small portions, shake gently and add to the polypropylene box. Shake 1 min and check pH with a moist pH paper inserted into the vapors of the polypropylene box. If not basic (pH 8), add 0.1 ml of collidine. Shake for 30 min and decant. Wash the Fmoc-peptidyl resin (5×DCM) (2×NMP). The Kaiser (ninhydrin) test should be negative (Step 4b, Scheme 1).
10. Coupling Fmoc-Glu(OtBu)-OH. Dissolve 0.42 g (0.99 mmol, 3.0 eq) of Fmoc–Glu(OtBu)-OH and 0.1 g (0.3 mmol, 1 eq) of BTC in 10 ml of DCM. Cool in an ice bath. Carefully add 0.37 ml (3.02 mmol, 8.4 eq) of collidine in small portions, shake gently and add to the polypropylene box. Shake 1 min and check pH with a moist pH paper inserted into the vapors of the polypropylene box. If not basic (pH 8), add 0.1 ml of collidine. Shake for 30 min and decant. Wash the Fmoc-peptidyl resin (5×DCM) (2×NMP). The Kaiser (ninhydrin) test should be negative (Step 4c, Scheme 1).
11. Fmoc deprotection. Put the three tea bags in a 100 ml polypropylene box. Remove the Fmoc protecting group by shaking with 20% piperidine/NMP (2×15 min). Wash the resin (3×NMP) (2×DCM). Monitor the deprotection by Kaiser (ninhydrin) test, which should be positive (Step 5, Scheme 1).
12. Cleavage of the peptide from the resin and OtBu deprotection. Wash the resin (2×DCM) (2×ether) and dry the tea bags under vacuum for at least 3 hr. Transfer the dry resin of each tea bag to a separate, marked polypropylene syringe (one can use a polypropylene centrifuge

tube) (Figure 5) and add 6 ml of a pre-cooled solution of TFA containing 1 drop of TDW and 1 drop of TIS to each syringe. Cool at 0ºC for 30 min and then shake for 90 min at rt. Remove the resin by filtration into a 50 ml polypropylene tube. In the hood, evaporate the solution by a stream of nitrogen for approximately 30 min to give about 2 ml oil (Step 6, Scheme 1).

13. Peptide precipitation. Treat the oil with cold ether (10 ml), shake vigorously (you may use a vortex), and allow the solid dipeptide to precipitate. Decant the ether layer and wash the solid again with cold ether (you may use centrifugation). Dry the solid in a vacuum desiccator for 30 min. Weigh the product and calculate the percent yield[11].
14. Purification and characterization. The crude product should be purified by HPLC and characterized by TLC, MS and NMR.

[11] If no solid precipitate is obtained, then remove the ether by reduced pressure and repeat the precipitation step.



Scheme 2 - Flowchart of the synthesis with "pause-points" marked (where the reaction can be safely paused between laboratory sessions, the tea bags may be refrigerated covered with DCM in a closed polypropylene box for no longer than one week).

Products

All products are white solids and were obtained in yields of 65-68%. TLCs were done in $CHCl_3$:CH_3OH:CH_3COOH (60:30:10), on silica.

H-Asp-Phe- NH_2

Yield, 50 mg (68%); TLC, R_f 0.55; ^1H NMR (CF_3COOD, 300 MHz, 298 K): δ_H(ppm) 2.69 (d, 2H), 2.83 (d, 2H), 4.25 (t, 1H), 4.48 (t, 1H) 6.69-6.80 (m, 5H) (Figure 6(a)). HPLC retention time = 28.003 min (Figure 6(b)). MS calculated for $C_{13}H_{17}N_3O_4$ 279.29, found 280.07 *m/z* $(M+H)^+$ (Figure 6(c)).

H-D-Asp-Phe-NH_2

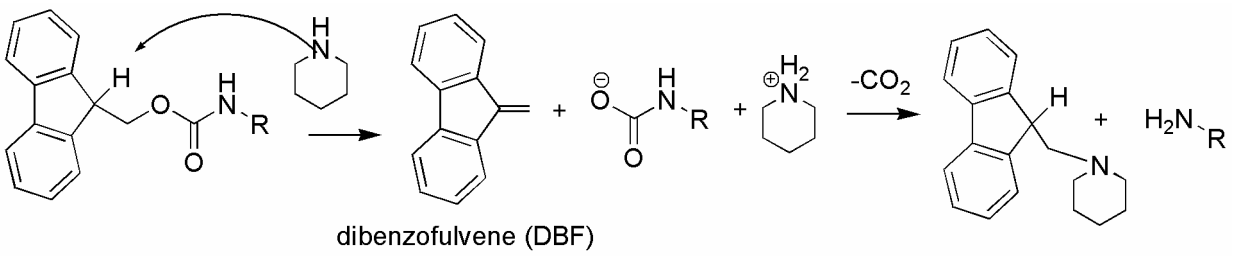
Yield, 48 mg (67%); TLC, R_f 0.55; ^1H NMR (CF_3COOD, 300 MHz, 298 K): δ_H(ppm) 2.69 (d, 2H), 2.83 (d, 2H), 4.25 (t, 1H), 4.48 (t, 1H) 6.69-6.80 (m, 5H). HPLC retention time = 27.950 min. MS calculated for $C_{13}H_{17}N_3O_4$ 279.29, found 280.27 *m/z* $(M+H)^+$.

H-Glu-Phe-NH_2

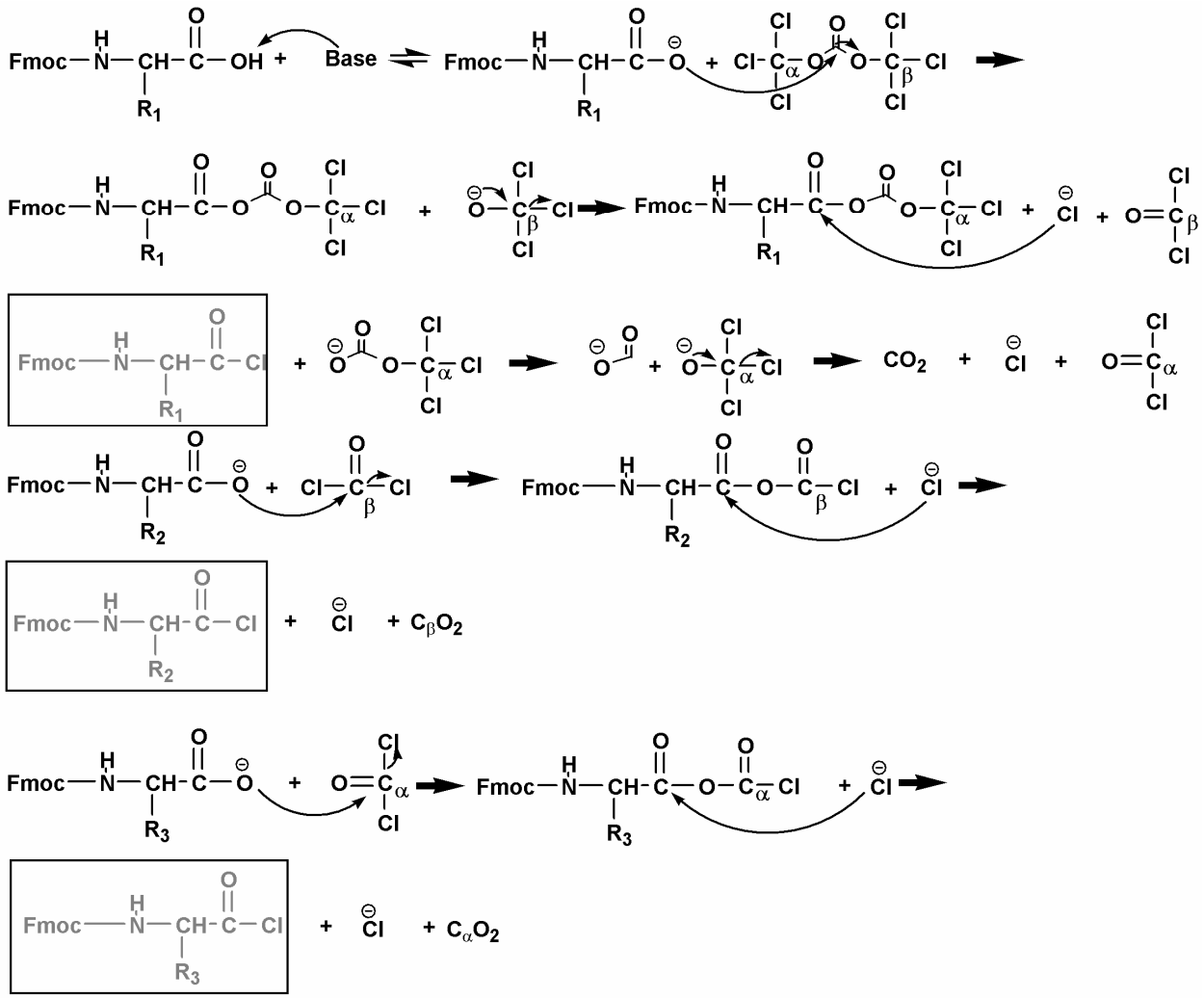
Yield, 45 mg (65%); TLC, R_f 0.65; ^1H NMR (CF_3COOD, 300 MHz, 298 K): δ_H(ppm) 2.69 (d, 2H), 2.73 (d, 2H), 2.83 (d, 2H), 4.25 (t, 1H), 4.48 (t, 1H) 6.69-6.80 (m, 5H). HPLC retention time = 28.030 min. MS calculated for $C_{14}H_{19}N_3O_4$ 293.32, found 294.60 *m/z* $(M+H)^+$.

Mechanism

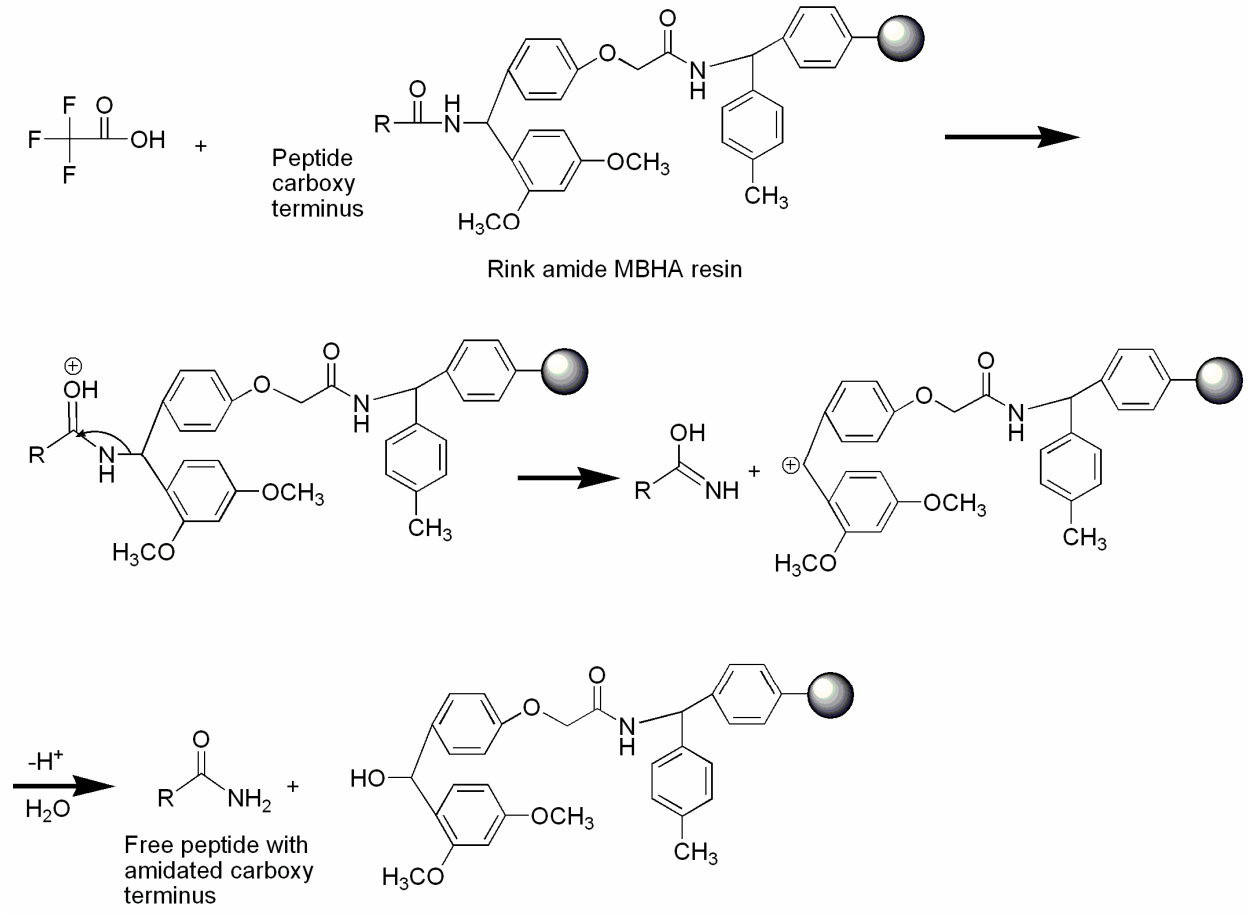
Mechanism for Fmoc deprotection



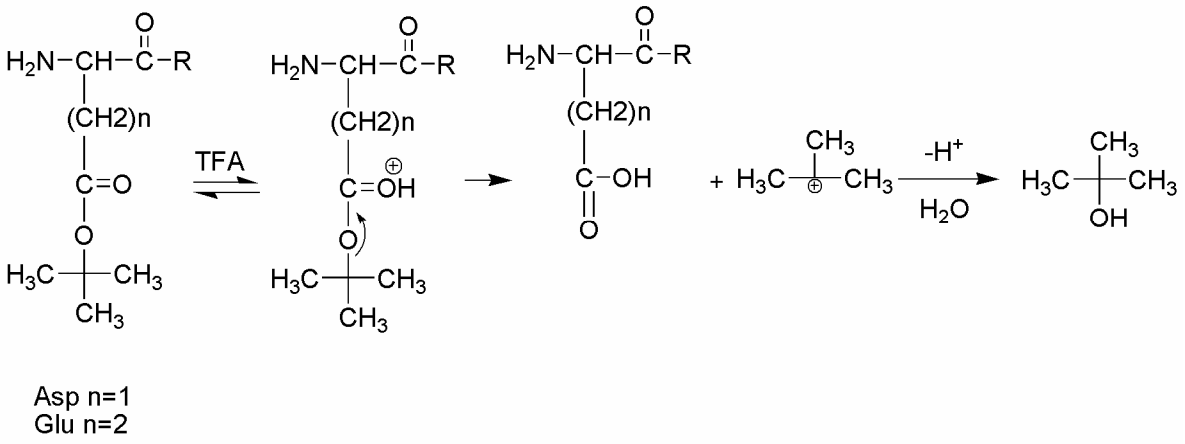
Mechanism of amino acid activation using BTC



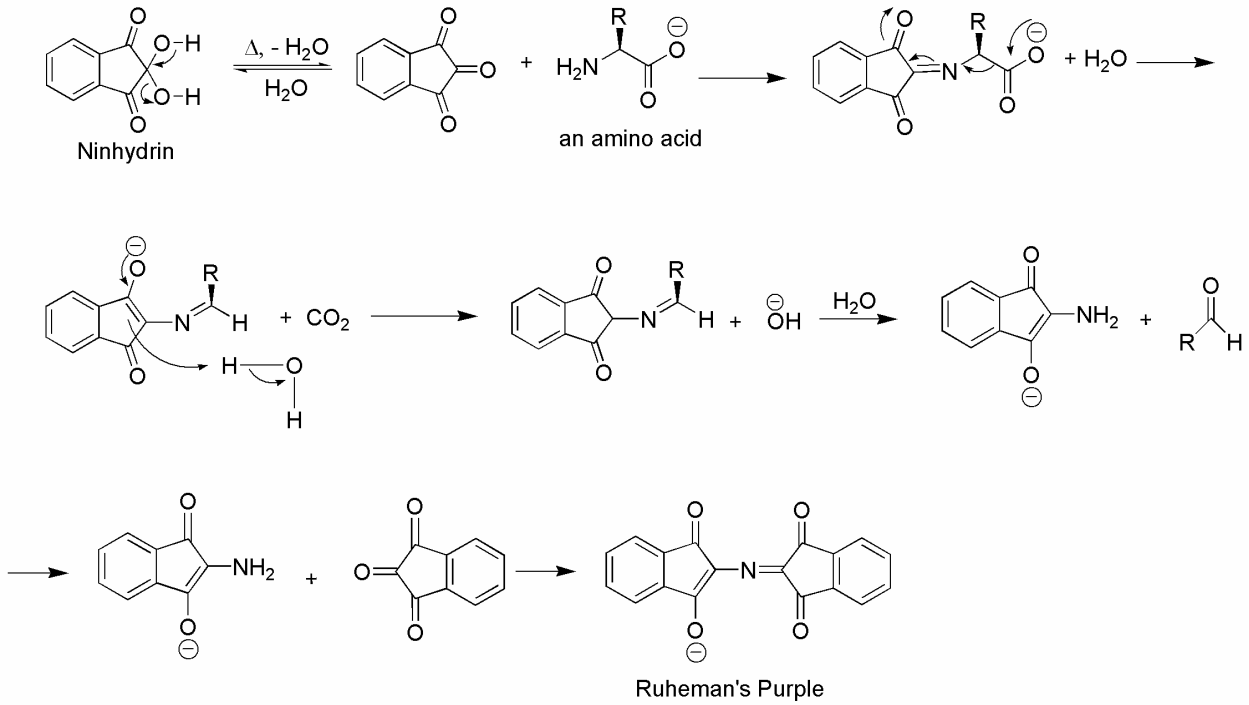
Mechanism of peptide cleavage from the resin



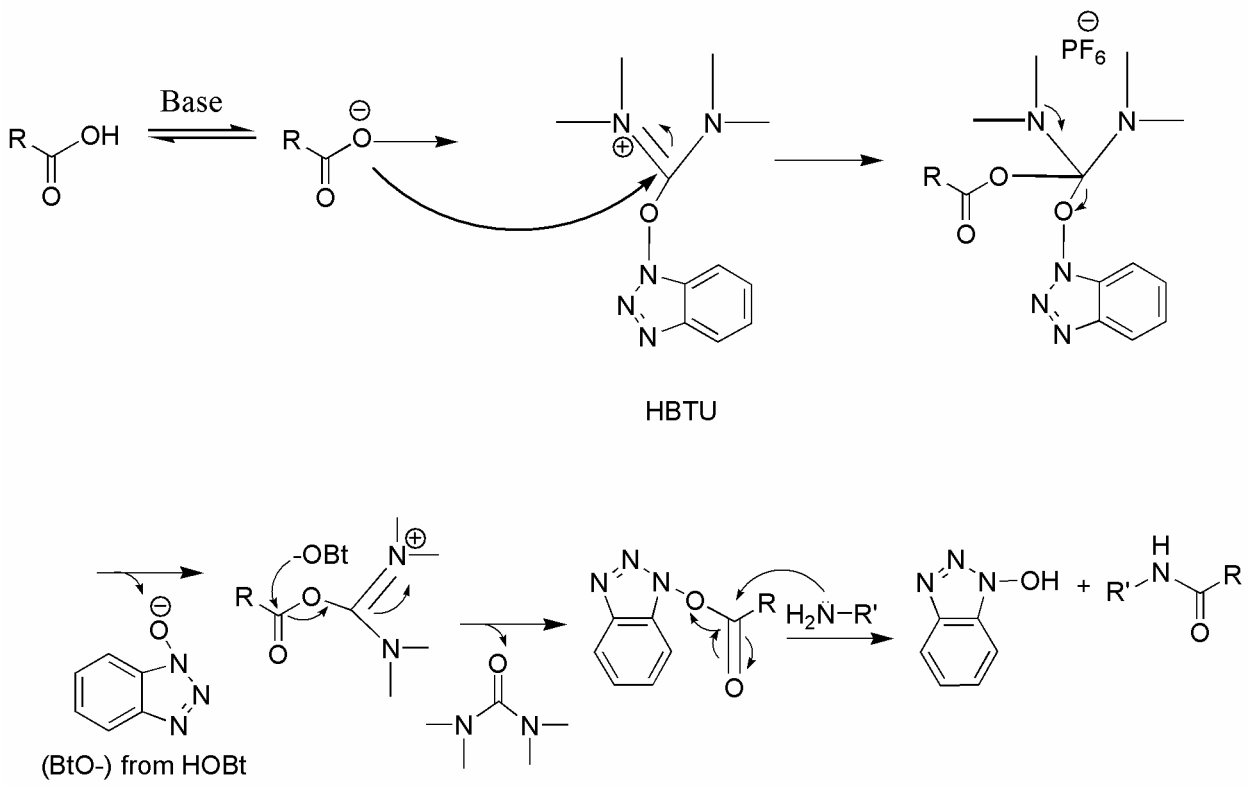
Mechanism of deprotection of the tBu protecting group from the Asp and Glu side chains



Mechanism of the Kaiser test (ninhydrin)



Mechanism of amino acid activation using HOBt/HBTU



Abbreviations

ACN	acetonitrile
Ala	alanine
Asp	aspartic acid
Boc	*t*-butoxycarbonyl
BTC	bis-(trichloromethyl)carbonate
ºC	degrees celsius
DBF	dibenzofulvene
DCM	dichloromethane
DIEA	diisopropylethylamine
DMF	dimethylformamide
DVB	divinylbenzene
eq	equivalent
FDA	Food and Drug Administration
Fmoc	9-fluorenylmethoxycarbonyl
g	gram
Gly	glycine
Glu	glutamic acid

HPLC	high-performance liquid chromatography
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3- tetramethylaminium hexafluorophosphate
HOBt	N-hydroxybenzotriazole
hr	hour
Lys	lysine
M	molar
mg	milligram
min	minutes
ml	milliliter
mm	millimeter
mmol	millimol
nm	nanometer
NMP	1-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
MHz	mega Hertz
MS	mass spectrometry
Phe	phenylalanine
ppm	parts per million

	polymeric solid support
R_f	$\text{TLC ratio} = \frac{\text{distance spot moved}}{\text{distance solvent moved}}$
rt	room temperature
SMPS	simultaneous multiple parallel synthesis
SPPS	solid phase peptide synthesis
tBu	*t*-butyl
TDW	triple distilled water
TFA	trifluoroacetic acid
TIS	triisopropylsilane
TLC	thin layer chromatography
TOF-MS	time of flight mass spectroscopy
Tyr	tyrosine
Uv	ultra violet
Vis	visible
μm	micrometer

Figure 1 - TEW electronic impulse auto-sealer.

Figure 2 – Shaker (Figure taken from St. John Associates, Inc. website, http://www.stjohnassociates.com/products/peptide_wrist-action_flask_shakers.htm, accessed June 2006).

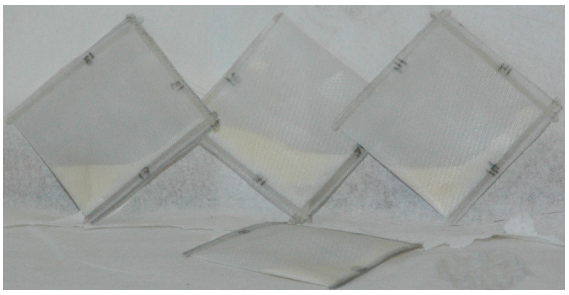


Figure 3 - Polypropylene bags.

Figure 4 - Polypropylene boxes (Figure taken from the Lab-supplies website,

http://lab-suppliesonline.com/essentials-bottles-square-c-3_173.html, accessed Jun 2006).

Figure 5 - Polypropylene syringes.

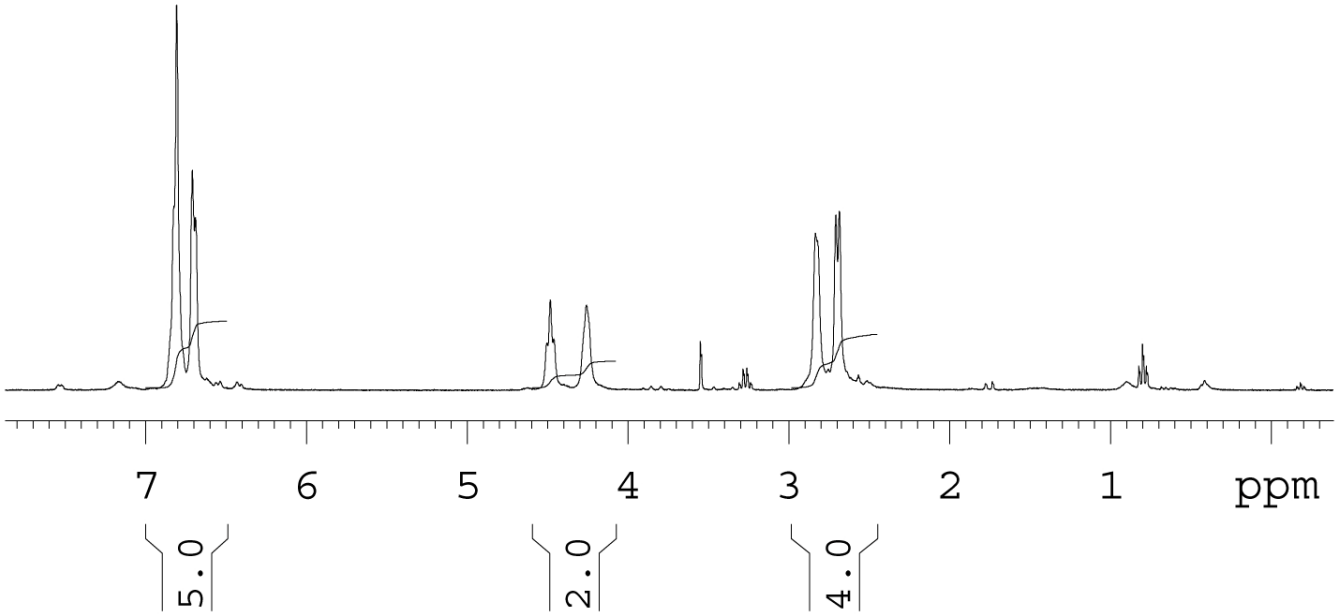


Figure 6 (a) – ^{1}H-NMR of H-Asp-Phe- NH_2 in TFA (300 MHz).

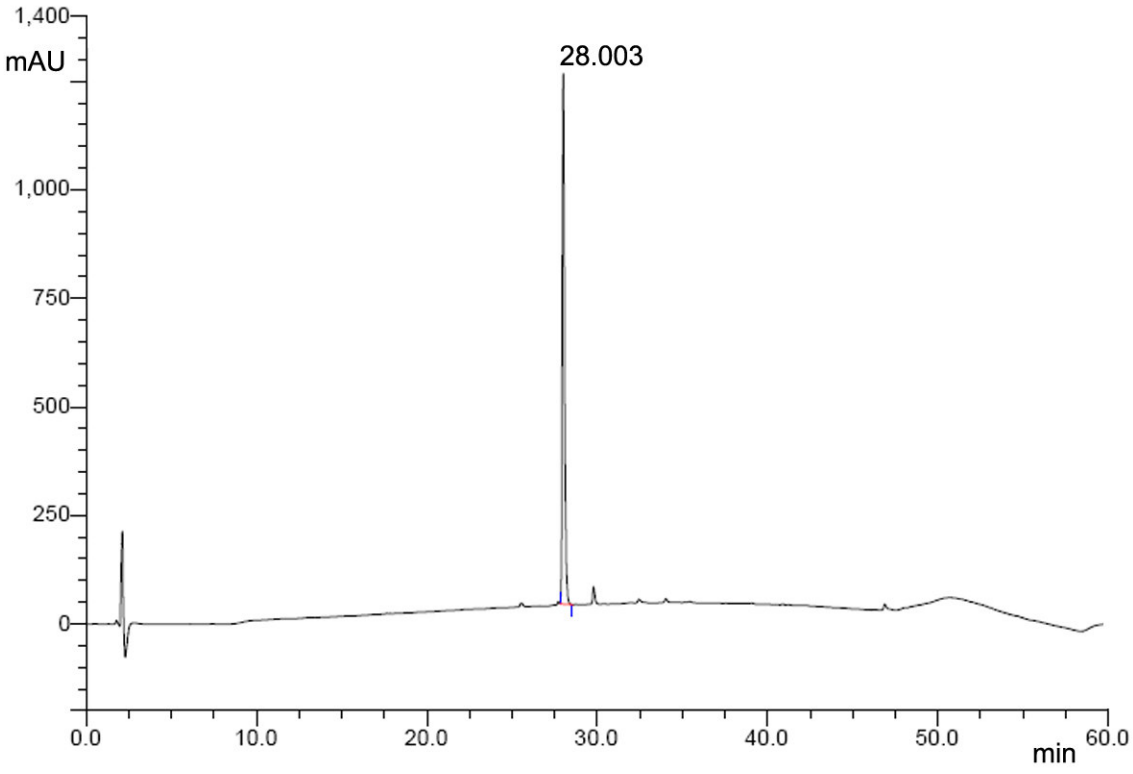


Figure 6 (b) - HPLC of H-Asp-Phe- NH_2.

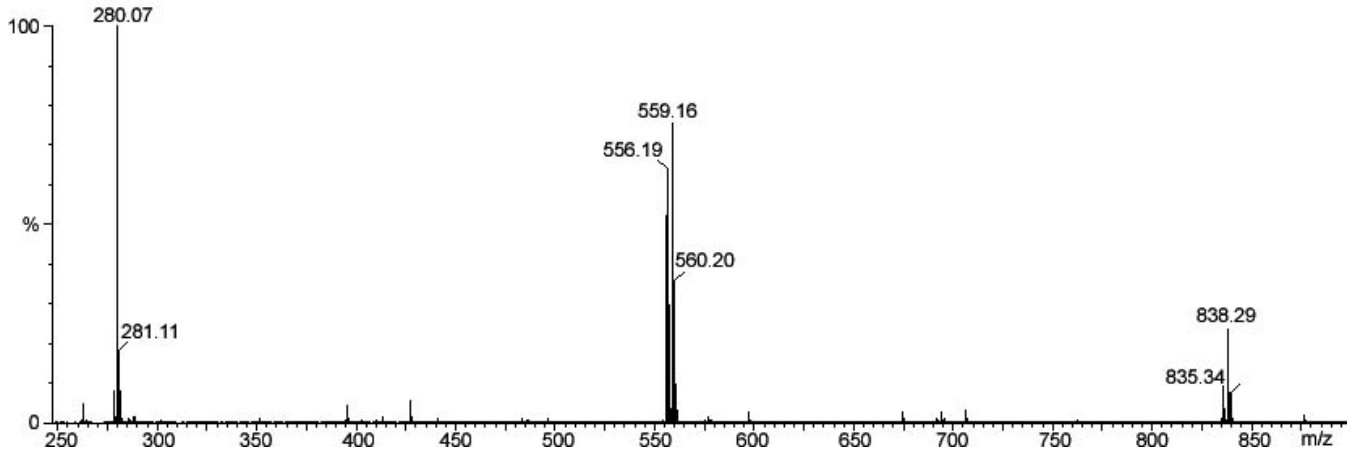


Figure 6 (c) – MS of H-Asp-Phe- NH_2 - The peaks are: 280.07 *m/z* $(M+H)^+$ - H-Asp-Phe-NH_2, 556.19 *m/z* - Fmoc-Asp-Phe-NH_2, 559.16 *m/z* – gas phase adduct of two molecules of H-Asp-Phe-NH_2, 835.34 *m/z* - gas phase adduct of Fmoc-Asp-Phe-NH_2 + Asp-Phe-NH_2 and 838.29 *m/z* - gas phase adduct of three molecules of H-Asp-Phe-NH_2.

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