

Introducing Freshmen Students to the Practice of Solid-Phase Synthesis

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Background and Rationale for the Experiment

Solid-phase chemistry has revolutionized the synthesis of organic compounds, peptides, oligonucleotides, carbohydrates, drugs, and combinatorial libraries (1–5). Although many types of compounds incorporating a variety of building blocks can be created, all solid-phase syntheses share a common approach. In a typical synthesis, starting material is anchored to a polymeric support and transformed into product by the action of solution-phase reagents. The product is then liberated into solution. The reverse approach has been used to convert solution-phase reactants into products by means of support-immobilized reagents and catalysts (6). Solid-phase organic synthesis is effective because reagents can be used in excess to drive reactions and immobilized products can be purified by repeated washings (7). In addition, syntheses can be automated. By combining these attractive features, multistep syntheses that would be considered unfeasible under homogeneous solution-phase conditions have been realized. The merit and popularity of solid-phase protocols are illustrated by the example of Bruce Merrifield, who received a Nobel Prize for his seminal contribution, and further, by explosive developments in combinatorial chemistry and drug discovery (5, 8–11).

Our goal was to introduce students in the early stages of academic development to the principles and practice of synthesis. Freshmen students attending a “pick-a-project” course were ideal subjects for the study. Several enrollees had expressed a curiosity in the topic of synthetic chemistry, but clearly lacked the practical experience required to carry out a multistep solution synthesis. As solid-phase methods have proven advantageous, popular, relevant, and technically facile, a project focused on solid-phase synthesis showed promise as an instructional vehicle. Peptide synthesis in particular was chosen because its chemistry has been developed to the point where success is virtually guaranteed if protocols are followed faithfully (2, 12–14). The novelty of presenting peptide chemistry to freshmen students in a tangible, hands-on fashion rather than by using a textbook approach was also a deciding factor (15).

Structure, Content, and Pedagogy

Students were asked to write a project proposal, present goals, rationale, and methods to a committee, implement the proposal, document results, and conclude the project in report and oral PowerPoint format before peers and faculty. In addition to instructor guidance and support, the students had intranet access to guidelines on preparing proposals, reports, and oral presentations and to mandatory readings on the subjects of synthesis and analysis (2, 12–18). Basic chromatography, spectrometry, spectroscopy, kinetics, and enzyme

function were discussed, and detailed protocols were supplied to facilitate in-lab instruction.

Project objectives were realized by adopting a guided, step-by-step, hands-on approach. The students worked and studied collaboratively in order to promote interactive learning. They were introduced to key concepts with the aid of laboratory demonstrations and visual tools and were encouraged to consolidate these concepts as they implemented the project. The completed project was archived on the intranet for the benefit of future enrollees.

Synthesis, Analysis, and Application

The peptides Bz-Asn-Asn-Phe and Bz-Asn-Gln-Phe were synthesized on a Wang resin (0.1 meq loading) using Fmoc chemistry (12, 14, 19, 20). The synthesis was performed using a capped vessel that was fitted with a filtration frit and fastened to a mechanical shaker. Chemicals were delivered by pipet and removed by suction. Each step from *a* to *c* (Fig. 1) was described by a condensation phase (0.5 mmol amino acid, 0.5 mmol *O*-benzotriazol-1-yl-*N,N,N'*-tetramethyluronium tetrafluoroborate [TBTU], 1 mmol diisopropylethylamine, and 2 mL dimethylformamide [DMF], 1–3 h), a wash phase (5 mL DMF, 4 × 2 min), and a deprotection phase (15 mL 20% piperidine–80% DMF, 15 min). After the resin was incubated in deprotection reagent, 10 μ L was

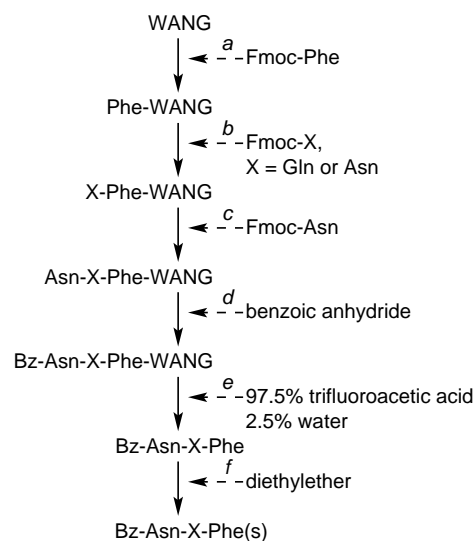


Figure 1. Solid-phase synthesis of target peptides. (a) Amino acid is anchored; (b, c) Peptide is elongated; (d) N-terminus is capped; (e) Peptide is liberated into solution; (f) Peptide is recovered by precipitation.

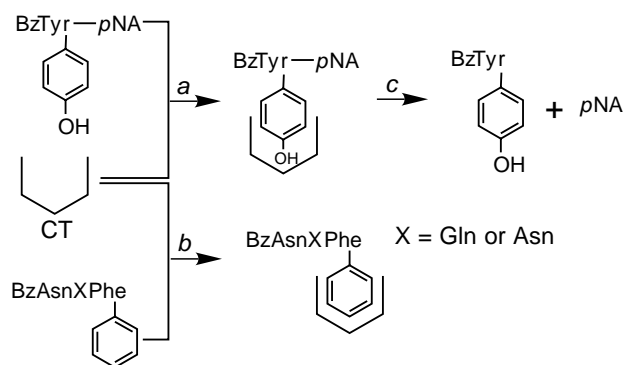


Figure 2. Mechanism proposed to explain the decreased rate of BzTyr-*p*NA hydrolysis following addition of BzAsnX Phe. Binding of (a) substrate (0.1 mM BzTyr-*p*NA) and (b) peptide (1 mM BzAsnX Phe) to chymotrypsin (CT, 3.3 μ g/mL in pH 7.5 buffer of the composition 50 mM Na phosphate, 5 mM KCl and 4.5% DMSO) is a dynamic process in which the enzyme-substrate population falls in response to a rise of BzAsnX Phe. Since only the enzyme-substrate complex proceeds onward to hydrolysis (c), the rate should decrease if BzAsnX Phe is introduced (22).

diluted (1:100) into fresh reagent and the dibenzylfulvene-piperidine adduct was quantified at 290 nm (14). By applying the principle of reaction stoichiometry, a yield for each condensation reaction was obtained. Yields were generally quantitative. To confirm that Fmoc was cleaved completely, the resin was washed and placed again in deprotection reagent. This time, a near-zero absorbance reading was observed. Benzoylation at step *d* (0.5 mmol benzoic anhydride, 1 mmol pyridine, and 5 mL CH₂Cl₂, 30 min) was confirmed by incubating a sample of resin in cadmium-ninhydrin solution (16). Acid-catalyzed cleavage at step *e* (5 mL 97.5% trifluoroacetic acid-2.5% water, 2.5 h) was achieved without adding foul-smelling scavengers such as ethanedithiol and thioanisole (14). The cleavage solution was reduced to 0.5 mL and 15 mL of ice-cold ether was added to precipitate the peptides (step *f*). Technicians verified product quality by using fast atom bombardment mass spectrometry and high-performance liquid chromatography.

Bz-Asn-Asn-Phe and Bz-Asn-Gln-Phe were employed as test peptides in the kinetic assay of chymotrypsin (18). Students first measured the rate of hydrolysis of BzTyr-*p*-nitroanilide (BzTyr-*p*NA), a chromogenic substrate. The assay was then repeated in the presence of Bz-Asn-Asn-Phe or Bz-Asn-Gln-Phe. Both peptides demonstrated a slight inhibitory effect on the rate of BzTyr-*p*NA hydrolysis. The mechanism of inhibition was rationalized using a competitive model for substrate binding (Fig. 2) (21, 22).

Hazards

Students should wear gloves and safety glasses, and perform their work inside a fume hood to prevent inhalation of vapors and powders. As trifluoroacetic acid is extremely irritating to the skin, eyes, and lung tissue, direct participation of the instructor is advisable at the cleavage step. If contact

occurs to the skin or eyes, flush the affected area immediately with water and irrigate for another 15 minutes. With single-eye injuries, pay close attention that water from the injured eye does not enter the good eye. Other chemical hazards are cadmium acetate and pyridine. Cadmium acetate is regarded as a potential OSHA carcinogen, and chronic exposure to pyridine can damage nerve tissue and reproductive function.

Concluding Remarks

The solid-phase peptide project was one of 26 projects that were offered to 110 enrollees of a course entitled Project 102. This project course was introduced to the Faculty of Engineering and Natural Sciences and Faculty of Arts and Social Sciences in the spirit of introducing freshmen students to teamwork. Seventeen faculty members supervised projects in different subject areas.

While it was stressed that obtaining data was secondary to the learning process, students expended substantial effort to achieve their goals. In the case of the solid-phase peptide project, the project team more than compensated for lack of experience with energy and initiative, and to their credit the students successfully completed all requirements. The chemistry employed was generally safe, permitting some supervision to take place from a distance. Mistakes were made and syntheses were repeated, but eventually, the students demonstrated not only competence in their technique but also confidence in their judgment. In particular, cheers filled the laboratory as soon as a peptide was precipitated out of ether. The students identified with the educational merit of carrying out manual syntheses, but also hinted, to our amusement, that automation would have been preferable. Moreover, they expressed surprise at being able to compare their achievements to those of third-year undergraduate students who conducted similar syntheses in solution (23).

While a detailed kinetic analysis was beyond the scope of the project, the students understood in principle that their observations reflected a dynamic interplay between peptide, substrate, and chymotrypsin. When reminded that acetyl-Phe is a competitive inhibitor of chymotrypsin (21, 22) and a "sub-unit" of both target peptides, they recognized the possibility that some peptide had bound to the enzyme in place of substrate (Fig. 2). They were also led to reason, as Bz-Asn-Asn-Phe was slightly more inhibiting than Bz-Asn-Gln-Phe, that subtle changes of structure could modulate binding of peptide to enzyme.

Of the three budding scientists who completed the peptide project, none had seriously considered a career in synthetic chemistry, and after completing the course their viewpoints had not changed. Nevertheless, they expressed gratitude at having been given the opportunity to dispel any second thoughts at a very early stage of their education.

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Supplemental Material

A student handout and notes for demonstrators are available in this issue of *JCE Online*.

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