Peptide and Peptide Nucleic Acid Syntheses Using a DNA/RNA Synthesizer

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ABSTRACT:

The use of an ABI 394 DNA/RNA synthesizer for peptide and peptide nucleic acid (PNA) syntheses is described. No additional physical part or software is needed for the application. A commercially available large DNA synthesis column was used, and only about half of its volume was filled with resin when the resin was fully swollen. With additional space in the top portion of the column, agitation of reaction mixture was achieved by bubbling argon from the bottom without losing solution. Removing solutions from column was achieved by flushing argon from top to bottom. Two peptide and two PNA sequences were synthesized. Good yields were obtained in all the cases. The method is easy to follow by researchers who are familiar with DNA/RNA synthesizer. © *2014 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 102: 487–493, 2014.*

Keywords: solid phase synthesis; synthesizer; DNA; RNA; peptide; peptide nucleic acid

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Additional Supporting Information may be found in the online version of this article.

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INTRODUCTION

Solid phase peptide and oligonucleotide (ON) syntheses are traditionally performed on different synthesizers. Such different designs are a result of several considerations. For example, the commonly used solvent for peptide synthesis is DMF, while that for ON is acetonitrile. The former has a higher viscosity than the latter, which causes different mechanical designs to drive liquid transfer. In addition, the number of monomer positions on the synthesizers is different. For peptide synthesizer, more than 20 are preferred, while for DNA/RNA synthesizer, 4–8 are enough. Furthermore, peptide synthesis is mostly performed at 1–100 mmol scales while ON synthesis is normally performed below 10 μmol. However, one of the most significant differences that cause different synthesizer designs is the kinetics of the reactions. The reactions for peptide synthesis are slow, while those for ON synthesis are fast. Because of this, peptide synthesis is usually performed batch-wise, and the reaction chamber on peptide synthesizers is a flask that can be shaken or a synthesis column through which the reaction solutions can be circulated. These designs are also beneficial to the usually larger scale peptide synthesis, where better agitation of reaction mixture and more efficient use of regents are desirable. In contrast, ON synthesis can be performed using a continuous-flow system, and the reaction chamber on a DNA/RNA synthesizer is usually a synthesis column without a circulation function. This design reduces instrument cost, and may be even more suitable for small scale synthesis compared to using a reaction flask, where small volumes of solutions may be used less efficiently. The ABI 394 DNA/RNA synthesizer adopted such a synthesis column design. Mainly due to this continuous-flow feature, DNA/RNA synthesizers are considered not suitable for peptide synthesis. However, there are good reasons to enable this function. Peptide synthesizers are expensive and take significant lab space. The use of DNA/RNA synthesizer for peptide synthesis will enable one instrument for two applications, which will save money on instrument and increase lab space

Table I Bottle Assignments and Amount of Reagents for Peptide Synthesis[a]

		Solution for One Cycle				
Bottles	Reagents/Solvents	Equivalent[b]	ml	mmol	Concentration (*M*)	Total (ml)
1–8	Amino acids in DMF	3	0.4	0.168	0.420	0.4
9	Blocked	—	—	—	—	—
10	DIEA/2,6-lutidine in DMF	3/3	0.4	0.168	0.420	[c]
11	Ac_2O in DMF for capping	10 × 3	0.6	0.560 × 3	0.935	[c]
12	Pyridine in DMF for capping	40 × 3	0.6	2.240 × 3	3.733	[c]
14	Piperidine in DMF for de-blocking	50 × 3	1.2	2.800 × 3	~20%	[c]
15	HATU in DMF for activating	2.8	0.4	0.156	0.392	[c]
18	DMF	—	—	—	—	[c]
19	DCM	—	—	—	—	[c]

[a] The synthesis was performed using 100 mg 2-chloro-trityl resin (0.56 mmol/g loading, 56 μmol).
[b] The synthesis column could retain 0.6 ml solution while bubble argon from bottom, but 1.2 ml (0.4 ml amino acid solution + 0.4 ml DIEA/lutidine solution + 0.4 ml HATU solution) solution of activated amino acid solution was delivered to improve volume accuracy. The actual equivalents of amino acids and activator used were therefore 1.5. The actual equivalents of capping and de-blocking reagents were also half of the values shown in table.
[c] Excess prepared.

usage. Furthermore, the conjugates of DNA/RNA with peptides are receiving increasing attention for biological studies.[1–3] The synthesis of such conjugates on a single synthesizer is expected to be more convenient. In this Report, we show that peptides and peptide nucleic acids (PNAs) can be synthesized on an ABI 394 DNA/RNA synthesizer. No additional physical part or software is needed. The procedures are easy to follow by anyone who is familiar with DNA synthesis, and good yields can be obtained in both cases.

EXPERIMENTAL

Determine Column Volume

The 2-chloro-trityl polystyrene resin (100 mg, 0.56 mmol/g loading, 56 μmol, 1% DVB, 100–200 mesh) was loaded in a 10–15 μmol DNA/RNA synthesis column (Glen Research, Sterling, VA), placed on an ABI 394 DNA/RNA synthesizer, and swollen in DCM for 30 min (see protocol 3 in Supporting Information). DCM was removed by reverse flushing, and the column was filled with DMF. To remove residue DCM, the DMF was removed by reverse flush with argon from the top of the column and the column was refilled with DMF. The column was then flushed with argon from bottom using the flush to waste function for 5 s. After waiting for 30 s, the flush was repeated. Because there was significant empty space within the top portion of the column after a portion of DMF was removed during the first flush and the column had a much bigger internal diameter than the argon port, not all DMF could be removed with the flushes. The bottom of the column was detached from the synthesizer, and the DMF remained in the column was pushed out into a beaker by reverse flush. The volume was determined to be about 0.6 ml.

Determine Times Required for Reverse Flush and Reagent Delivery

Reagents and solvents were placed on the synthesizer according to Table I (see protocol 3 in Supporting Information). The column containing swollen resin and filled with DMF was flushed from the top to record the time for emptying the column by reverse flush. The time was recorded in Table II (entry 1). The time required for filling the column with DMF was also recorded (entry 2). For measuring the times needed to add 0.4 ml base solution (from bottle 10, entry 3) and 0.4 ml activator solution (from bottle 15, entry 4) to amino acid bottles 1–8, the solutions were first pressurized and the lines from the bottles (10 and 15) to block were filled. The block and the lines to the amino acid bottles were cleaned and emptied. The amino acid bottle is removed. A graduated cylinder (or graduated 1.5 ml centrifuge tube) is placed under the liquid line. Then, the solution from either bottle 10 or 15 was delivered to the cylinder for an arbitrarily chosen time using an appropriate function (e.g., 10–5, 15–5) followed by cleaning up the block (18 to waste, block flush) and flushing the liquid in the line between the block and amino acid bottle to the cylinder (e.g., flush to 5). These need to be tested several times until the correct time for delivering 0.4 ml solution is determined. The times for delivering activated amino acid (entry 5) and capping (entry 6) and de-blocking solutions (entry 7) to column were measured by first empty the column followed by appropriate delivery functions shown in Table II.

Table II Times for Reverse Flush and Reagent Deliveries for Peptide Synthesis[a]

Entry	Function Name/Function Number	Time (s)
1	Reverse flush/2	30
2	18 (DMF) to column/42	35
3	10 (bases) to amino acid/for example, 216	4.2
4	15 (activator) to amino acid/for example, 200	10.5
5	1–8 (amino acid) to column/33	38
6	11+12 (capping solutions) to column/39	35
7	14 (de-blocking solution) to column/40	35

[a] Argon pressure for regulators 1–3 on the synthesizer were 6.3, 6.3, and 8.6 psi, respectively.

Synthesis of Peptide 1

The six-amino acid peptide **1** (LWTRFA) was synthesized on a 56 μmol scale using 100 mg resin (2-chloro-trityl polystyrene with Ala attached, loading 0.56 mmol/g, 1% DVB, 100–200 mesh; see protocol 1 in Supporting Information). The resin was placed in a commercially available 10/15 μmol ON synthesis column. The Fmoc protected amino acids Phe, Arg(Pbf), Thr(tBu), Trp(Boc), and Leu were dissolved in DMF and placed in bottles 1–5 on the synthesizer, respectively (see Table I for reagent amounts). HATU was used as activator and placed in bottle 15. The acid generated during activation was neutralized with diisopropylethylamine (DIEA) and 2,6-lutidine, which were placed in bottle 14. Capping failure sequences were achieved with acetic anhydride and pyridine from bottles 11–12. Fmoc removal was achieved with piperidine from bottle 14. The reagents and times for reverse flush and reagent delivery are summarized in Tables I and II. Using these data, the begin (Supporting Information Table S3), synthesis (Supporting Information Table S1), and end cycles (Supporting Information Table S4) were composed (see Supporting Information), and the synthesis was set up accordingly (see protocol 1 in Supporting Information). For attaching amino acid bottles, ensure that the lines from block to the bottles are empty after bottle change (a suggested cycle to achieve this is in Supporting Information Table S2). For attaching other bottles, ensure that the lines from block to them are filled after bottle change. This is especially important for bottles 10 and 15. The sequence was edited as 5TCGA for Leu, Trp(Boc), Thr(tBu), Arg(Pbf), and Phe, respectively (note, the synthesis starts from A to 5). For setting up synthesis, select appropriate begin cycle (Supporting Information Table S3), synthesis cycle (Supporting Information Table S1), column, sequence, and end cycle (Supporting Information Table S4). The cycles are in Supporting Information.

Cleavage, Deprotection, and Analysis of Peptide 1

A portion of the resin (30 mg) was taken from the column and swollen with DCM for 30 min. The DCM was removed and the resin was treated with a TFA cocktail containing 81.5% TFA, 1.0% triisopropylsilane (TIPS), 5.0% water, 2.5% ethane dithiol (EDT), 5.0% thioanisole, and 5.0% phenol (300 μl) at rt for 100 min. The supernatant was removed, and the resin was washed with TFA cocktail (100 μl $\times$ 2). The peptide in the combined solutions was precipitated by cold ether (4.5 ml) at -78°C. The supernatant was removed. The crude peptide was dissolved in 1.2 ml 50% acetonitrile, and 20 μl was injected to RP HPLC to generate trace A (Figure 1). The peak at 23 min was collected and one-third was re-analyzed with HPLC to generate trace B. The total pure peptide from the 30 mg resin was determined to be 10.7 mg, which is a white pellet. The yield was calculated to be 80% based on the resin loading value provided by the manufacturer. The peptide was analyzed with ESI-MS, calcd for $C_{39}H_{57}N_{10}O_8$ $[M+H]^+$ 793.4, found 793.4 (see Supporting Information).

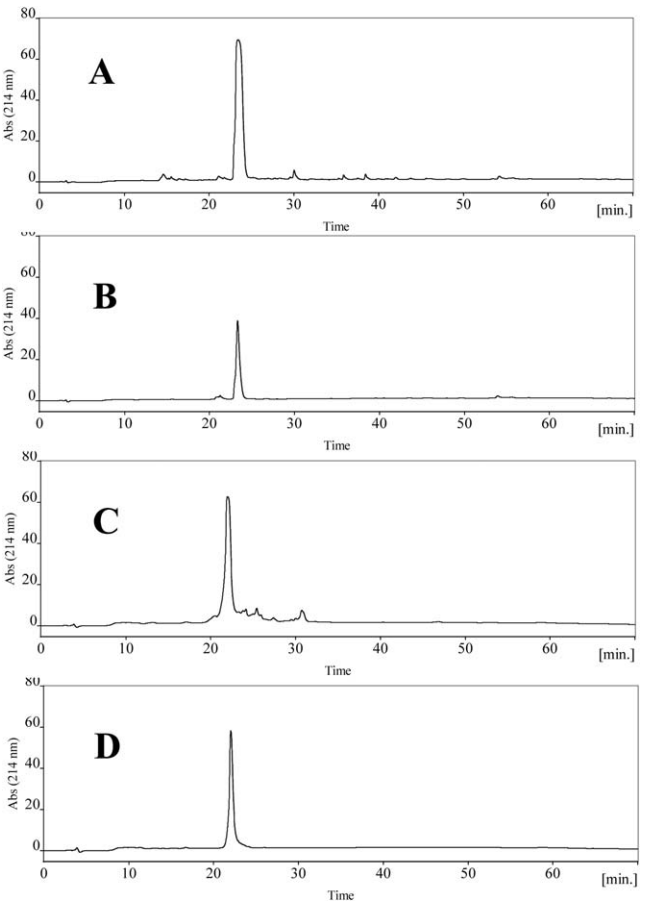

FIGURE 1 RP HPLC profiles: (a) Crude peptide **1**. (b) Pure peptide **1**. (c) Crude peptide **2**. (d) Pure peptide **2**.

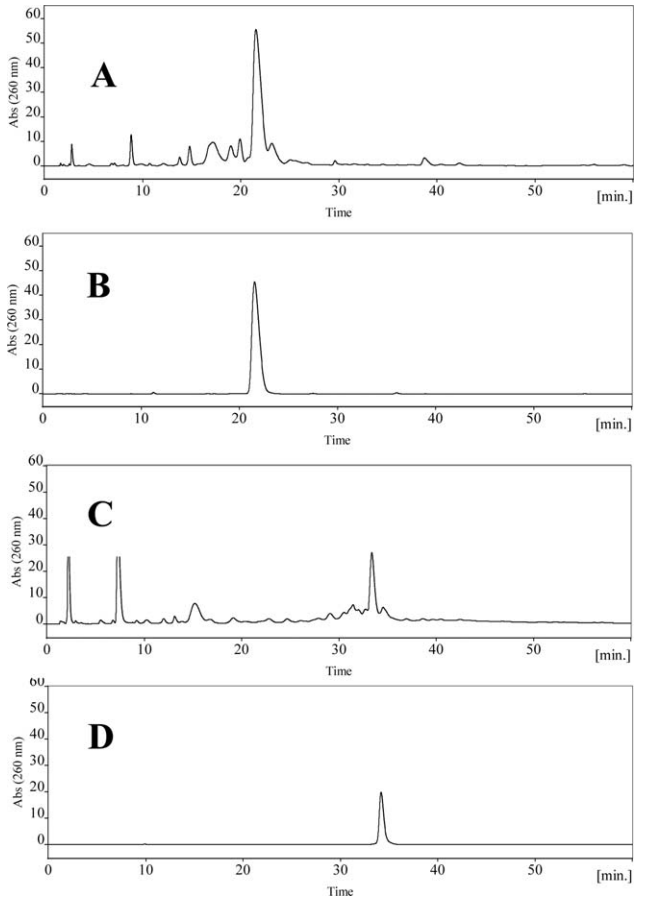

FIGURE 2 RP HPLC profiles: (a) Crude PNA **3**. (b) Pure PNA **3**. (c) Crude PNA **4**. (d) Pure PNA **4**.

Synthesis, Cleavage/Deprotection, and Analysis of Peptide 2

The 16-amino acid peptide **2** (KNRWEDPGKQLYNVEA) was synthesized under the same conditions for **1** at 56 μmol (100 mg resin) scale using the Fmoc amino acid monomers Glu(OtBu), Val, Asn(Trt), Tyr(tBu), Leu, Gln(Trt), Lys(Boc), Gly, Pro, Asp(OtBu), Trp(Boc), and Arg(Pbf). First, an 8-mer was synthesized, and the resin (the synthesis cycle contains washing steps that make resin ready for next synthesis) was then used directly for adding the remaining eight amino acid monomers on the synthesizer. After the synthesis is complete, a portion of the resin (35 mg) was taken from the column. Cleavage and deprotection were achieved under the same conditions for **1**. The crude peptide was dissolved in 1.3 ml 50% acetonitrile, and 20 μl was injected to RP HPLC to generate trace C (Figure 1). The peak at 22 min was collected and re-analyzed with HPLC to generate trace D. The total pure peptide **2**, which is a white pellet, from the 35 mg resin was 11.7 mg. The yield was calculated to be 89% based on the resin loading value provided by the manufacturer. The pure peptide **2** was analyzed with ESI-MS, calcd for $C_{86}H_{132}N_{25}O_{26}$ $[M+H]^+$ 1948.1, found 1947.6 (see Supporting Information).

Synthesis, Cleavage/Deprotection, and Analysis of PNA 3

The 5-mer PNA **3** (H_2N-TAGAC-$CONH_2$) was synthesized on a 56 μmol scale (90 mg Sieber amide resin, 0.62 mmol/g loading, 1% DVB, 100–200 mesh; see protocol 2 in Supporting Information) using *N*-(*N*-Fmoc-2-aminoethyl)-*N*-[(*N*-6-Bhoc-9-adenyl)acetyl]-glycine, *N*-(*N*-Fmoc-2-aminoethyl)-*N*-[(*N*-4-Bhoc-1-cytosyl)acetyl]-glycine, *N*-(*N*-Fmoc-2-aminoethyl)-*N*-[(*N*-6-Bhoc-9-guanyl)acetyl]-glycine, and *N*-(*N*-Fmoc-2-aminoethyl)-*N*-[(1-thyminylacetyl]-glycine as the monomers. The reagents and solvents were placed on the synthesizer as in Table III. The times for reverse flushing column and reagent delivery were determined as described for peptide, and listed in Table IV (see protocol 4 in Supporting Information). These data were used for composing the begin (Supporting Information Table S6), synthesis (Supporting Information Table S5), and end cycles (Supporting Information Table S7). The synthesis was set up in the same way for peptide (see protocol 2 in Supporting Information). After synthesis, a portion of the resin (6 mg) was incubated with TFA containing *m*-cresol (9:1, v/v, 50 μl) at rt for 30 min. The supernatant was removed by filtration, and the step was repeated for four more times. The cleaving solutions were combined. The PNA was precipitated with cold ether (1.5 ml) at -20°C. The supernatant was removed by filtration. The residue was dissolved in 80 μl water; 10 μl was injected into RP HPLC to generate trace A (Figure 2). The peak at 21 min was collected, concentrated, re-dissolved in 20 μl 50% acetonitrile, and injected to HPLC to generate trace B. The total pure PNA from 6.0 mg resin was determined to be 2.1 mg, which is a white pellet. The yield was calculated to be 75% based on the resin loading value provided by the manufacturer. The pure PNA was analyzed with ESI-MS, calcd for $C_{54}H_{70}N_{31}O_{14}$ $[M+H]^+$ 1376.6, found 1376.6 (see Supporting Information).

Synthesis, Cleavage/Deprotection, and Analysis of PNA 4

The 10-mer PNA **4** (H_2N-CTGCTTAGAC-$CONH_2$) was synthesized under the same conditions for **3** at 56 μmol (90 mg resin) scale using the same monomers used for **3**. First, a 5-mer was synthesized. A small portion of the resin (~0.3 mg) was taken out, and subjected to cleavage and deprotection using conditions for **3**. ESI-MS indicated that the synthesis was successful. The remaining resin was used directly for adding the remaining five monomers on the synthesizer. After the synthesis is complete, a portion of the resin (5 mg) was taken

Table III Bottle Assignments and Amount of Reagents for PNA Synthesis[a]

		Solution for One Cycle				
Bottle	Reagents/Solvents	Equivalent[b]	ml	mmol	Concentration (*M*)	Total (ml)
1–8	Amino acids in NMP	2	0.4	0.112	0.280	0.4
9	Blocked	—	—	—	—	—
10	DIEA/2,6-lutidine in NMP	2/2	0.4	0.112	0.280	[c]
11	Ac_2O in NMP for capping	10 × 3	0.6	0.560 × 3	0.933	[c]
12	Pyridine in NMP for capping	40 × 3	0.6	2.240 × 3	3.733	[c]
14	Piperidine in DMF	50 × 3	1.2	2.800 × 3	∼20%	[c]
15	HATU in NMP for activating	2	0.4	0.112	0.280	[c]
18	DMF	—	—	—	—	—
19	DCM	—	—	—	—	—

[a] The synthesis was performed using 90 mg Sieber amide resin (0.62 mmol/g loading, 56 μmol).
[b] The synthesis column could retain 0.6 ml solution while bubble argon from bottom, but 1.2 ml (0.4 ml amino acid solution + 0.4 ml DIEA/lutidine solution + 0.4 ml HATU solution) solution of activated amino acid solution was delivered to improve volume accuracy. The actual equivalents of amino acids and activator used were therefore ∼1.0. The actual equivalents of capping and de-blocking reagents were also about half of the values shown.
[c] Excess prepared.

from the column. Cleavage and deprotection were achieved under the same conditions for **3**. The residue was dissolved in 80 μl water; 10 μl was injected into RP HPLC to generate the trace C (Figure 2). The peak at 33 min was collected, evaporated, and re-dissolved in 20 μl water, and injected to HPLC to generate the trace D. The total pure PNA **4**, which is a white pellet, from the 5 mg resin was 1.9 mg. The yield was calculated to be 61% based on the resin loading value provided by the manufacturer. The pure PNA was analyzed with ESI-MS, calcd for $C_{107}H_{138}N_{56}O_{31}$ $[M+2H]^{2+}$ 1351.6, found 1351.8 (see Supporting Information).

RESULTS AND DISCUSSION

To use the ABI 394 synthesizer for peptide synthesis, a method to agitate the reaction mixture must be identified. Potential solutions to solve the problem include installation of a circulation pump and performing the reactions in a flask with a filtering function. However, these solutions are complicated. We envisioned that simply using a larger DNA synthesis column and only filling about half of the column space with resin when it is swollen would solve the problem. With empty space over the resin, agitation of the reaction mixture can be carried out by an argon flow from the bottom of the column (the flush to waste function) without losing the reaction solution. Removal of reaction solution and washing solvents can be achieved by an argon flow from the top of the column (the reverse flush function).

Table IV Times for Reverse Flush and Reagent Deliveries for PNA Synthesis[a]

Entry	Function Name/Number	Time (s)
1	Reverse flush/2	40
2	18 (DMF) to column/42	40
3	10 (bases) to amino acid/for example, 216	8
4	15 (activator) to amino acid/for example, 200	18
5	1–8 (amino acid) to column/33	80
6	11+12 (capping solutions) to column/39	50
7	14 (de-blocking solution) to column/40	40

[a] Argon pressure for regulators 1–3 on the synthesizer were 6.3, 6.3, and 8.6 psi, respectively.

With a solution for agitation in mind, the next issues to address are the following. (i) Assign bottles for amino acids and other reagents. We used the option shown in Table I. (ii) Determine the volume of DMF that can remain in a column containing swollen resin after bubbling argon from the bottom. This can be measured by filling the column with DMF, bubbling argon from bottom, detaching the bottom of the column from synthesizer, and flushing the remaining DMF from the top by argon into a graduated cylinder (see protocol 3 in Supporting Information). (iii) Determine the volume and concentration of solutions of amino acids, bases, activator, capping, and de-blocking. This can be calculated using the column volume, the desired equivalents of reagents, and the total loading of the first amino acid on resin. (iv) Determine the times required for filling the column with DMF, removal of DMF in column by reverse flush, adding base and activator solutions to amino acid bottles, delivering activated amino acids to column, and delivering capping and de-blocking solutions to column (see Materials and Methods section). These are better to be tested using the real solutions instead of DMF

because they have different viscosity. In our experiments, those for activator (bottle 15) and base (bottle 10) to amino acid bottles (e.g., bottle 5) were measured using real solutions because delivering accurate volumes of these solutions is critical for the success of synthesis. For others, delivering more than needed only consumes more reagents but the synthesis will not be affected. As a result, we used DMF for the tests even though real solutions are suggested in protocol 3. (v) Edit new functions. They are for adding activator and bases to amino acids (each eight functions for bottles 1–8), and for agitating the solutions in bottles 1–8 while activating the amino acids by an argon flow (see Supporting Information Table S8). (vi) Compose begin, synthesis, and end cycles. This can only be accomplished with the times required for reverse flushing column and reagent deliveries, and the new functions. The begin cycle includes steps for filling lines to bottles 10–12 and 14–15, and for removing the Fmoc group on the resin if there is one. The synthesis cycle mainly includes steps for activating amino acids, delivering activated amino acids to column, agitating the reaction mixture in column, washing column, adding capping solutions, and de-blocking solution to column. The end cycle includes steps for washing the resin and the lines from block to the amino acid bottles. With these issues addressed, a peptide (or PNA) synthesis can be conveniently set up.

To test feasibility, the six-amino acid peptide (**1**, LWTRFA) was synthesized on a 56 μmol scale using 100 mg resin (loading 0.56 mmol/g; see protocol 1 in Supporting Information). The 2-chloro-trityl polystyrene resin with Ala attached was placed in a normal 10/15 μmol ON synthesis column taking about half of the total volume after swelling. The Fmoc protected amino acids Phe, Arg(Pbf), Thr(tBu), Trp(Boc), and Leu were dissolved in DMF and placed in bottles 1–5 on the synthesizer, respectively. HATU was used to activate the amino acids. The acid generated during activation was neutralized with DIEA and 2,6-lutidine. Capping failure sequences were achieved with acetic anhydride and pyridine. Piperidine was used for Fmoc removal. The reagents and times for reverse flush and reagent delivery are shown in Tables I and II. Using these data, the begin, synthesis, and end cycles were composed (see Supporting Information), and the synthesis was set up accordingly. After synthesis, a portion of the resin was taken out from the column. Cleavage and deprotection were performed with a TFA cocktail containing 81.5% TFA, 1.0% triisopropylsilane (TIPS), 5.0% water, 2.5% ethane dithiol (EDT), 5.0% thioanisole, and 5.0% phenol. The peptide was precipitated with cold ether, and analyzed with RP HPLC. As shown in Figure 1, trace A contains a major peak at 23 min, which is resulted from peptide **1**. The smaller peaks around 14 min and 21 min are mainly from failure sequences that are capped with acetic anhydride. The profile of purified **1** is also shown (trace B). The isolated yield of pure peptide was calculated to be 80%, and its structure was identified with ESI-MS (see Supporting Information). To further demonstrate the technique, the 16-amino acid peptide **2** (KNRWEDPGKQLYNVEA) was synthesized and cleaved under the same conditions. The crude peptide was analyzed with RP HPLC. As shown in Figure 1 trace C, the synthesis was very good considering the length of the peptide and the low equivalents of amino acid monomers we used. The peptide was easily purified (trace D). The isolated yield was calculated to be 89%, and the structure was identified with ESI-MS (see Supporting Information).

To use the method for PNA synthesis, some parameters need to be modified (Tables III and IV). Because of the much higher price of PNA monomers than Fmoc amino acids, we reduced their equivalents to two, which corresponds to approximately one because about half of the activated monomers could not be retained in the synthesis column during bubbling. For PNA synthesis, the coupling and capping steps are normally performed in NMP instead of DMF. Because the NMP solutions have higher viscosity, the times for delivering these solutions and reverse flushing them out of column have to be longer. Based on these considerations, we re-tested all the parameters (see protocol 4 in Supporting Information), and used them to modify the begin (Supporting Information Table S6), synthesis (Supporting Information Table S5), and end cycles (Supporting Information Table S7). To test feasibility, the PNA sequence (H_2N-TAGAC-$CONH_2$, **3**) was synthesized on a 56 μmol scale using the Sieber amide resin (see protocol 2 in Supporting Information). At the end of synthesis, a portion of the resin was taken out from column and treated with a TFA solution containing *m*-cresol. The cleaved and fully deprotected PNA was precipitated with cold ether, and analyzed with RP HPLC (trace A, Figure 2). The results indicated that the synthesis was successful. The full-length PNA appeared at 21 min. Other peaks were mainly failure sequences. The profile for purified **3** is shown in trace B. The isolated yield was calculated to be 75%, and its structure was identified with ESI-MS (see Supporting Information). To further demonstrate the technique, the 10-mer PNA **4** (H_2N-CTGCTTAGAC-$CONH_2$) was synthesized and cleaved under the same conditions. The crude PNA was analyzed with RP HPLC (trace C, Figure 2). The isolated yield was calculated to be 61%, which could be easily improved by increasing the equivalents of monomers. The product was readily purified with RP HPLC (trace D, Figure 2). Its structure was identified with ESI-MS (see Supporting Information).

Because the 394 synthesizer has eight nucleoside phosphoramidite bottles, the synthesis of peptides and PNAs shorter than 9-mer (the first amino acid is usually attached to resin

before automated synthesis) can be achieved without any interruption. Synthesis of longer sequences is also not complicated. It was easily achieved by starting a new synthesis using the resin of nine-amino acid oligomer. For peptide, sequences longer than 40-mer are rarely synthesized using automated synthesizer continuously. For PNA, the most commonly used length is around 12-mer. As a result, the limited number of monomer positions on the DNA/RNA synthesizer is not a concern for the technique. Normally, continuous synthesis of long peptides and PNAs should be avoided in most cases and tests such as ESI-MS, Kaiser, and TNBS should be performed to monitor the efficiency of coupling.

In the above synthesis examples, only about half of the activated amino acid solution was retained in the column after bubbling argon from bottom. This can be considered as a waste of reagents. We could have reduced the volume of amino acid, base, and activator solutions to solve the problem, but mixing smaller volumes in a precise ratio had been predicted more difficult. However, we were able to partially solve the problem by inserting a long waiting time between delivering most of the solution to the column and delivering the remaining solution, the latter was followed by agitating the reaction mixture by bubbling argon (see the synthesis cycles in Supporting Information). A better way to solve the problem is to use a larger synthesis column or to reduce the synthesis scale.

CONCLUSION

In conclusion, we have developed a method for the synthesis of peptide and PNA using an ABI 394 DNA synthesizer. It is remarkable that no modification of the synthesizer is needed, and the only item needed for the method is a larger DNA synthesis column, which is commercially available and reasonably priced. No knowledge in areas such as mechanics and electronics is required. Anyone who is familiar with the DNA synthesizer can easily follow the procedures, and can set up the synthesizer in about 1 day. Once set up, peptide and PNA synthesis may be even more convenient than using some models of peptide synthesizer. The method is expected to be useful in labs that need peptides, PNA, and their conjugates with ONs.[4–6]

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