User Bulletin No. 33

Model 431A Peptide Synthesizer

November 1990 (updated 09/2002)

SUBJECT: *FastMoc™* Chemistry: HBTU Activation in Peptide Synthesis on the Model 431A

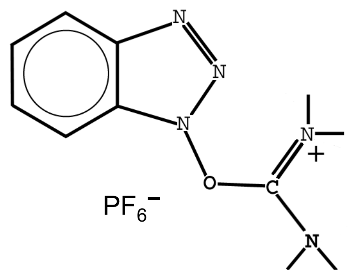

Figure 1 HBTU

HBTU [2-(1H-benzotriazol-l-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] is a new agent for coupling amino acids with Fmoc/NMP chemistry that is easily adapted for use on the Applied Biosystems Model 431A Peptide Synthesizer. Activation with HBTU is much faster and more complete than carbodiimide-mediated reactions and results in shorter cycle times and increased coupling efficiency.[1,2]

In the *FastMoc™* chemistry procedure developed at Applied Biosystems, HBTU is dissolved in a solution of HOBt (1-hydroxybenzotriazole) and DMF (dimethlyformamide). The amino acid is dissolved in this solution with additional NMP (N-methylpyrrolidone). This solution is then transferred directly to the reaction vessel. DIEA (diisopropylethylamine) is added to initiate the *in situ* reaction. With this procedure, even difficult couplings can be completed in 30 minutes or less.[3]

It has been observed that, in general, couplings are much faster with the *FastMoc™* chemistry. For example, short peptides (<15 amino acids) and simple peptides can be synthesized with coupling times of only 10 minutes on the 0.1 mmol scale. HBTU activation is recommended when difficult couplings are predicted, such as with Arg and the branched side chains of Ile, Val, and Leu.

This User Bulletin explains how to prepare reagents and the Model 431A Peptide Synthesizer for HBTU activation with Fmoc/NMP chemistry procedures. It describes new cycles for coupling, double coupling and deprotection with both the 0.25 mmol and the 0.10 mmol scales.

WARNING Chemicals used in the following procedures may be hazardous. When handling them, wear suitable protective clothing, chemical resistant rubber gloves, and use them only with adequate ventilation in a chemical fume hood.

Material Safety Data Sheets (MSDSs) are provided by Applied Biosystems or by the manufacturer or distributor of each chemical. MSDSs give information on physical characteristics, hazards, precautions, first aid, spill clean-up and disposal procedures. Please familiarize yourself with information in the MSDSs before handling or disposing of these reagents.

Contents

Synthesis Set-Up: 0.25 and 0.10 mmol scale

To set up *FastMoc*™ synthesis, follow the standard preparation for synthesis described in the Model 431A User's Manual, with the exceptions listed below. This User's Bulletin describes these changes in detail.

Bottle Changes Two bottles must be changed for the *FastMoc*™ chemistry. Replace Bottle 5 with 0.45 M HBTU/HOBt/DMF and replace Bottle 7 with DIEA. Follow the bottle change routine for Bottle 4, described in Chapter 1, Conversion of Fmoc and Boc Chemistries, in the User's Manual.

Flow Tests Use Flow Test 10, which delivers NMP from bottle 10 to the metering vessel, to set the lower regulator. Run Flow Tests 1, 9, 11, and 12 to check for clogged filters, insufficient seals, etc.

DO NOT RUN FLOW TEST 5! In Flow Test 5 the metering vessel is rinsed with DCM after the delivery. When this Flow Test is used with HBTU, an HBTU precipitate will collect in the vessel.

Use Module H to test the flow from Bottle 5 to the cartridge. The 9-second delivery should yield 2.1–2.3 grams (2.0–2.2 mL) of solution. If this delivery is off by more than 0.1 gram, adjust the delivery time both in module H and in module A.

When using unloaded HMP resins, run Flow Test 4 and Flow Test 8 before synthesis. DMAP and DCC are used infrequently with *FastMoc*™ chemistry. Use the flow tests to confirm that the delivery lines are clear.

Finally, run Flow Test 7 to clear the metering loop of any remaining DIEA.

Run Editor *FastMoc*™ modules must be used for the *FastMoc*™ chemistry. Set up the cycles as described later in the following pages of this bulletin. Pay particular attention to the use of lower and upper case letters. The chemistry in the *FastMoc*™ cycles is very different from the DCC/HOBt chemistry used in the standard Fmoc cycles.

Reagents and Bottle Positions

As Table 1 illustrates, you only need to change reagent bottles 5 and 7 to prepare the synthesizer for HBTU activation.

Table 1: Reagents and Solvents for Fmoc Chemistry with HBTU Activation on the Model 431A

Bottle #	Reagent	Part Number
1	Piperidine	400629
2	———	
4	0.1 M Dimethylaminopyridine in N,N,–Dimethylformamide	400631
5	0.45 M HBTU/HOBt in DMF (must be prepared)	401132
6	Methanol	400470
7	Diisopropylethylamine	400136
8	1.0 M Dicyclohexylcarbodiimide in N–Methylpyrrolidone	400663
9	Dichloromethane	400142
10	N–Methylpyrrolidone	400580

Bottle 5 (HBTU) Preparation

HBTU is purchased as part of the HBTU activation kit (P/N 401132). The activation kit contains a bottle of solid HBTU (100 mmol), a bottle of 0.5 M HOBt in DMF and two HBTU delivery line filters. Store the reagents in the HBTU activation kit at 0–4° C until you are ready to use them. Mix the reagents together just prior to use.

To prepare 0.45 M HBTU/HOBt/DMF:

Note Do not attempt to use the 1 M HOBt/NMP for this preparaton. HBTU does not readily dissolve in NMP at this concentration.

1. Pour 200 mL of 0.5 M 1-hydroxybenzotriazole in N,N,–dimethylformamide into the 450 mL bottle containing 100 mmol dry HBTU.
2. Dissolve the HBTU with gentle swirling. The increased volume due to HBTU reduces the concentration of the two species from 0.5 M to 0.45 M.
3. Locate the Bottle 5 delivery line and thoroughly dry it with a lint-free tissue.
4. Firmly press the polyethylene delivery line filter onto the end of the Bottle 5 delivery line. Screw Bottle 5 into the ratchet cap on the peptide synthesizer.

The 0.45 M HBTU/HOBt/DMF solution is stable at room temperature for at least 6 weeks, as determined by use testing. After a few days, the solution turns yellow. This color change does not have any adverse affect on the efficiency of the reagent.

Required Amino Acid Derivatives

The coupling efficiencies and solubilities of Fmoc-Gln and Fmoc-Asn are greatly enhanced when the side chains are protected. Based on their coupling performance and ease of removal during TFA cleavage, we recommend the trityl protected derivatives. Fmoc-Asn(Trt) and Fmoc-Gln(Trt) can also be used with the standard loading cycles to load HMP resin.

	Part Number (1 mmol cartridge)	
Amino Acid	each	box of 10
N–α–Fmoc–(trityl)–L–asparagine	401089	411089
N–α–Fmoc–(trityl)–L–glutamine	401090	411090

All other amino acids used in this procedure are the recommended, standard derivatives sold by Applied Biosystems.

Loading and Capping

When loading amino acids on HMP resin, run Flow Test 4 and Flow Test 8 before synthesis. Since DMAP and DCC are used infrequently with *FastMoc*™ chemistry, the reagent bottles must be pressurized and the delivery lines flushed with reagent. Accurate deliveries of these two reagents are essential for efficient loading.

A minor side reaction may be observed with *FastMoc*™ chemistry when loading is not efficient. HBTU–activated amino acid can react with some of the remaining hydroxyl sites on the HMP resin, producing peptides with deletions at the C-terminus that appear as small impurities in chromatograms and mass spectra.

Note If commercially-prepared, loaded resins are used, the hydroxyl sites have been capped.

If you find these minor impurities unacceptable, we recommend that the loading to HMP resin be followed by capping with benzoic anhydride. This is easily accomplished by filling a dry, clean cartridge with 2 mmol (0.45 g) benzoic anhydride and using it with the standard loading protocols. This procedure is described in “Examples for Run Editor”.

Required User Functions

To use the *FastMoc*™ chemistry on the Model 431A, you must define several User Functions. The 0.25 mmol cycles require one User Function and the 0.1 mmol cycles require four User Functions. Directions for writing User Functions can be found in Chapter 7, the Function Editor Menu, of the 431A User's Manual. You must re-enter User Functions each time the instrument is reset.

User Function for 0.25 mmol cycles:

Function #	Function Description	Valves Activated
94: User Fxn A	#5 to Cartridge	12, 15, 27, 30

User Functions for 0.10 mmol cycles:

Function #	Function Description	Valves Activated
94: User Fxn A	#5 to Cartridge	12, 15, 27, 30
95: User Fxn B	Vent Measuring Loop	6, 13
96: User Fxn C	Cartridge to Closed Reaction Vessel	10, 12, 17, 22
97: User Fxn D	#7 to Closed Measuring Loop	30, 31

Flow Test for the HBTU Solution

During the synthesis, from 2.1 to 2.3 g of the 0.45 M HBTU/HOBt/DMF solution in bottle 5 should be delivered to the amino acid cartridge. You must write a flow test so that you can determine if this addition takes 8, 9, or 10 seconds. Write the flow test in Module H, shown below.

Module H

Step #	Fxn #	Function Name	Time	Add
1	5	NEEDLE DWN	10	0
2	10	GAS B VB	2	0
3	78	PRS #M	15	0
4	94	USER FXN A	9*	0
5	60	MIX CART	5	0
6	61	VENT CART	3	0
7	6	NEEDLE UP	10	0
8	7	EJECT CART	10	0
9	8	ADVAN CART	10	0
10	10	GAS B VB	10	0

*Step 4 is Fxn 94 (User Fxn A). This User Function activates valves: 12, 15, 27 and 30.

To use the Module H flow test, set the Run Editor as follows:

Cy: 1 Rpt: 1 M: H

Place a tared cartridge on the instrument when you run this flow test. After running Module H, weigh the solution in the cartridge.

Adjust the time in step 4 so that 2.1 to 2.3 g of the 0.45 M HBTU solution is delivered. You must also enter this adjusted time value in step 12 of Module A for the 0.25 mmol cycles and in step 7 of Module A for the 0.10 mmol cycles.

FastMoc™ Protocols

The 0.25 mmol *FastMoc*™ cycles use 1.0 mmol Fmoc-amino acids in the standard scale (40 mL) reaction vessel. The 0.10 mmol *FastMoc*™ cycles use the 1.0 mmol amino acid cartridge in the small scale (8 mL) reaction vessel. Tables 2 and 3 summarize the protocols for both scales on the Model 431A.

Table 2: Model 431A *FastMoc*™ Chain Assembly

Operation and Reagents	Time (minutes)	
	0.25 mmol cycles	0.10 mmol cycles
Deprotection	13	7
Washes with NMP	6	2
Coupling	30	9
Washes with NMP	6	2
Resin Sample (optional)	(2)	(6)
Total time	55*	20*

*Resin sampling adds 2 more minutes to the total cycle time of the 0.25 mmol cycles and 6 minutes to the total cycle time of the 0.10 cycles.

Table 3: Comparison of Model 431A *FastMoc*™ cycles

	Resin (mmol)	Amino Acid (mmol)	AA:Resin	Reaction vessel	Waste per cycle (mL)
0.25 mmol scale	0.25	1.00	4:1	Standard	160
0.10 mmol scale	0.10	1.00	10:1	Small Scale	50

FastMoc™ Chemical Usage

Table 4 summarizes the chemical usage of the 0.25 mmol and 0.10 mmol *FastMoc*™ cycles.

Table 4: Chemical Usage of 0.25 mmol and 0.10 mmol *FastMoc*™ Cycles

			0.25 mmol cycles		0.10 mmol cycles	
Bottle	Chemical	mL per Bottle	mL per Cycle	Cycles/Bottle	mL per Cycle	Cycles/Bottle
1	Piperidine	200	4.5	41	2.0	100
2	—	—	—	—	—	—
4	DMAP	200	†	†	†	†
5	HBTU/HOBt	230	2.2	104	2.2	104
6	MeOH	450	†	†	†	†
7	DIEA	175	0.5	350	0.5	350
8	DCC	200	†	†	†	†
9	DCM	4000	25	160	‡	—
10	NMP	4000	128	29	45	89
Waste	—	9463	160*	59	50*	189

† There is one delivery of DMAP (0.4 mL), one delivery of DCC (0.5 mL), and one delivery of MeOH (7 mL) per loading cycle.

‡ DCM is used only twice during a peptide synthesis with 0.10 mmol cycles: once when the first amino acid is loaded and then 15 mL is used in the final wash.

* This value is for non-resin-sampling cycles. Resin-sampling cycles produce 15 mL more waste per cycle.

Standard Scale (0.25 mmol)

The 0.25 mmol *FastMoc*™ cycles are used with either the resin sampling or non-resin sampling standard reaction vessel. (Refer to Chapter 1 of the 431A Users Manual regarding usage of resin sampling and non-resin sampling reaction vessels.) The Fmoc-amino acid is dissolved with 2.1 g NMP and 2.2 g of 0.45 M HBTU/HOBt in DMF. DIEA (ca. 0.22 g or 1.7 eq) is added during the transfer step (module E) to the reaction vessel.

FastMoc™ Module Descriptions (0.25 mmol)

Module A – Dissolution of amino acid **Total time = 7 minutes**

At the beginning of module A, the amino acid cartridge name is printed on the synthesis report, the old cartridge is ejected and the new cartridge is advanced. NMP (2.1 g) and 0.45 M HBTU/HOBt in DMF (2.2 g) are added to the cartridge. The amino acid is dissolved by mixing for 6 minutes. During this time, the second phase of the deprotection occurs.

Module B – Piperidine deprotection **Total time = 6 minutes**

This module begins with one NMP wash of the resin. A 20% piperidine/NMP solution is introduced and allowed to react for 3 minutes. After a second introduction of the mixture, the valve blocks are rinsed thoroughly.

Module D – NMP washes **Total time = 6 minutes**

The RV is drained and the resin is washed 5 times with NMP. With resin-sampling cycles, the resin-sampling line is rinsed 2 times. The cartridge is mixed intermittently for 3 seconds at a time.

Module E – Transfer and washing **Total time = 5 minutes**

The amino acid/HBTU/HOBt solution is tranferred to the reaction vessel, after which 0.3–0.34 mL DIEA is added. The cartridge and valve blocks are washed thoroughly. Approximately 3 minutes of coupling occurs at the end of this module.

Module F – Coupling **Total time = 25 minutes**

During this module, the reaction vessel is vortexed. For resin-sampling cycles, the resin-sampling line is cleared. Since this module alone determines 90% of the coupling time, the time can be easily customized.

Module G – Resin Sampling **Total time = 6 minutes**

The RV is drained and the resin is washed. For resin-sampling cycles, a resin sample is taken. Place a blank tube between each resin sample tube.

Module H – Flow Test for HBTU in bottle 5

Note This module is not for use in a synthesis!

This flow test delivers 2.0–2.2 mL 0.45 M HBTU/HOBt/DMF to the cartridge in approximately 9 seconds (see page 6, Flow Test for the HBTU Solution). This test should be done after Flow Tests 10 and 11 have been performed, using NMP, to set the lower regulator. Descriptions of these flow tests are found in the Model 431A Users Manual.

FastMoc™ Modules for the Run Editor (0.25 mmol)

For every amino acid that is to be coupled, the cycle should have the following series of modules:

BADEFG

If preloaded resins or C-terminal amide resins are used, this cycle should be used for the first and subsequent cycles. Note that this order of modules differs from the standard scale DCC/HOBt Fmoc cycles. In the *FastMoc*™ cycles, there is no pre-activation of the Fmoc-amino acid and module A is used for dissolving. For this reason, module A appears after the deprotection (module B) has begun.

If an amino acid requires double-coupling, that cycle should have these modules:

BADEFAGEFG

Use two amino acid cartridges for each double couple.

If you need to load the C-terminal amino acid to an HMP resin, the first cycle should be:

hefffg

and all subsequent cycles:

BADEFG.

This combination of cycles will leave the Fmoc group on the peptide-resin.

If an unprotected peptide is desired, the final cycle should be:

bdc.

If you want to load the C-terminal amino acid to an HMP resin and follow this with a capping step, the first cycle should be:

hefffghefffg.

The first cartridge is your amino acid and the second cartridge contain 2.0 mmol (0.45 g) benzoic anhydride.

If you are loading His or Arg, refer to the Loading Modifications section in Chapter 5 of the Model 431A Users Manual. Table 5, below, summarizes the cycles used for the *FastMoc*™ chemistry.

Table 5: Cycles Used with *FastMoc*™ Chemistry (0.25 mmol)

Cycle type	Modules	Cycle time (min)
Loading and Capping	hefffghefffg	198
Coupling	BADEFG	55
Double couple	BADEFAGEFG	98
N–terminal deprotection	bdc	36

Examples for Run Editor (0.25 mmol)

Example 1: Angiotensin, a 10-mer peptide

To synthesize Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu on HMP resin, with removal of the final Fmoc group:

Cy: 1	Rpt: 1	M: hefffg
Cy: 2	Rpt: 9	M: BADEFG
Cy: 11	Rpt: 1	M: bdc

Example 2: Angiotensin, a 10-mer peptide

To synthesize Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu on HMP resin with capping after loading and removal of final Fmoc group:

Cy: 1	Rpt: 1	M: hefffghefffg
Cy: 2	Rpt: 9	M: BADEFG
Cy: 11	Rpt: 1	M: bdc

Example 3: Angiotensin, a 10-mer peptide

To synthesize Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu starting with Fmoc-Leu-resin, with removal of the final Fmoc group:

Cy: 1	Rpt: 9	M: BADEFG
Cy: 10	Rpt: 1	M: bdc

Example 4: Angiotensin, a 10-mer peptide

To synthesize Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu starting with Fmoc-Leu-resin, and the final Fmoc group remains on the peptide-resin:

Cy: 1	Rpt: 9	M: BADEFG
Cy: 10	Rpt: 1	M: c

Example 5: Substance P, an 11-mer amide peptide

To synthesize Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2 on Rink resin, with removal of the final Fmoc group:

Cy: 1	Rpt: 11	M: BADEFG
Cy: 12	Rpt: 1	M: bdc

0.25 mmol *FastMoc*™ Modules

Module A

Step #	Fxn #	Function Name	Time	Add
1	4	PRINT CART	10	0
2	6	NEEDLE UP	10	0
3	7	EJECT CART	10	0
4	8	ADVAN CART	10	0
5	5	NEEDLE DWN	10	0
6	14	#10 B VB	2	0
7	9	GAS T VB	2	0
8	65	#10 CART	5	0
9	60	MIX CART	5	0
10	78	PRS #M	10	0
11	18	#5 B VB	1	0
12*	**94**	**USER FXN A**	**9****	0
13	98	BEGIN LOOP	6	0
14	2	VORTEX ON	1	0
15	60	MIX CART	30	0
16	3	VORTEX OFF	1	0
17	60	MIX CART	30	0
18	99	END LOOP	1	0

* Step 12 is Fxn 94 (User Fxn A). This user-defined function activates the following valves: 12, 15, 27 and 30.

** The value for the time in step 12 is determined by running module H, and varies slightly from instrument to instrument. We have used either 8, 9, or 10 sec. in our development work. The objective is to have 2.1 to 2.3 g of the 0.45 M HBTU solution delivered to the cartridge.

Module B

1. Copy Std Fmoc mod b to mod B.
2. Delete steps 35 to 62 (28 deletions).
3. Delete step 10.
4. Add the following steps at the end:

Step#	Fxn#	Function Name	Time	Add
34	2	VORTEX ON	1	0
35	14	#10 B VB	3	0
36	9	GAS T VB	2	0
37	12	#9 B VB	3	0
38	9	GAS T VB	2	0
39	10	GAS B VB	10	0
40	3	VORTEX OFF	1	0

Module b:

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	56	#10 B RV	15	1
3	88	RS TO RV	1	0
4	91	#10 TO RS	5	0
5	41	VENT RV	2	0
6	89	RS TO FC	1	0
7	2	VORTEX ON	5	0
8	40	MIX RV	3	0
9	10	GAS B VB	6	0
~~10~~	~~20~~	~~MIX ACT~~	~~1~~	~~0~~
11	3	VORTEX OFF	1	0
12	42	DRAIN RV	15	1
13	58	#10 B RV	13	2
14	79	PRS #1	10	0
15	51	#1 B RV	8	1
16	56	#10 B RV	2	0
17	40	MIX RV	3	0
18	98	BEGIN LOOP	6	0
19	2	VORTEX ON	15	0
20	3	VORTEX OFF	15	0
21	99	END LOOP	1	0
22	79	PRS #1	5	0
23	41	VENT RV	3	0
24	42	DRAIN RV	25	1
25	56	#10 B RV	8	1
26	51	#1 B RV	10	1
27	56	#10 B RV	8	1
28	40	MIX RV	3	0
29	41	VENT RV	2	0
30	93	GAS TO RS	3	0
31	88	RS TO RV	1	0
32	93	GAS TO RS	3	0
33	41	VENT RV	2	0
34	89	RS TO FC	1	0
35	9	GAS T VB	3	0
36	10	GAS B VB	3	0
37	98	BEGIN LOOP	14	0
38	2	VORTEX ON	15	0
39	3	VORTEX OFF	15	0
40	99	END LOOP	1	0
41	41	VENT RV	2	0
42	88	RS TO RV	1	0
43	93	GAS TO RS	3	0
44	41	VENT RV	2	0
45	89	RS TO FC	1	0
46	98	BEGIN LOOP	14	0
47	2	VORTEX ON	15	0
48	3	VORTEX OFF	15	0
49	99	END LOOP	1	0
50	41	VENT RV	2	0
51	88	RS TO RV	1	0
52	91	#10 TO RS	5	0
53	89	RS TO FC	1	0
54	50	#10 RV-DRN	5	0
55	42	DRAIN RV	20	0
56	56	#10 B RV	27	1
57	40	MIX RV	2	1
58	2	VORTEX ON	5	0
59	3	VORTEX OFF	2	0
60	42	DRAIN RV	20	1
61	50	#10 RV-DRN	5	0
62	42	DRAIN RV	10	0

Module : B

431A Version : 1.12 Fmoc

Date / Time : 06/08/90 11:41

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	56	#10 B RV	15	1
3	88	RS TO RV	1	0
4	91	#10 TO RS	5	0
5	41	VENT RV	2	0
6	89	RS TO FC	1	0
7	2	VORTEX ON	5	0
8	40	MIX RV	3	0
9	10	GAS B VB	6	0
10	3	VORTEX OFF	1	0
11	42	DRAIN RV	15	1
12	56	#10 B RV	13	2
13	79	PRS #1	10	0
14	51	#1 B RV	8	1
15	56	#10 B RV	2	0
16	40	MIX RV	3	0
17	98	BEGIN LOOP	6	0
18	2	VORTEX ON	15	0
19	3	VORTEX OFF	15	0
20	99	END LOOP	1	0
21	79	PRS #1	5	0
22	41	VENT RV	3	0
23	42	DRAIN RV	25	1
24	56	#10 B RV	8	1
25	51	#1 B RV	10	1
26	56	#10 B RV	8	1
27	40	MIX RV	3	0
28	41	VENT RV	2	0
29	93	GAS TO RS	3	0
30	88	RS TO RV	1	0
31	93	GAS TO RS	3	0
32	41	VENT RV	2	0
33	89	RS TO FC	1	0
34	2	VORTEX ON	1	0
35	14	#10 V VB	3	0
36	9	GAS T VB	2	0
37	12	#9 B VB	3	0
38	9	GAS T VB	2	0
39	10	GAS B VB	10	0
40	3	VORTEX OFF	1	0

Module D

1. Copy Std Fmoc mod d to mod D.
2. Change step 38 to Fxn 60 for 3 sec.
3. Change step 22 to Fxn 60 for 3 sec.
4. Delete steps 11 to 13 (3 deletions).
5. Delete steps 2 to 4 (3 deletions).

Module : D
431A Version : 1.12 Fmoc
Date / Time : 06/08/90 11:42

Module d:

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
~~2~~	~~55~~	~~#10 B RV~~	~~12~~	~~1~~
~~3~~	~~2~~	~~VORTEX ON~~	~~1~~	~~0~~
~~4~~	~~40~~	~~MIX RV~~	~~2~~	~~0~~
5	41	VENT RV	1	0
6	88	RS TO RV	1	0
7	93	GAS TO RS	3	0
8	91	#10 TO RS	5	0
9	89	RS TO FC	1	0
10	40	MIX RV	2	0
~~11~~	~~10~~	~~GAS B VB~~	~~6~~	~~0~~
~~12~~	~~20~~	~~MIX ACT~~	~~2~~	~~0~~
~~13~~	~~3~~	~~VORTEX OFF~~	~~1~~	~~0~~
14	42	DRAIN RV	10	1
15	50	#10 RV-DRN	5	0
16	42	DRAIN RV	10	0
17	98	BEGIN LOOP	4	0
18	56	#10 B RV	13	1
19	2	VORTEX ON	1	0
20	40	MIX RV	3	0
21	10	GAS B VB	6	0
22	~~20~~ 60	MIX ~~ACT~~ CART	~~1~~3	0
23	3	VORTEX OFF	1	0
24	42	DRAIN RV	10	1
25	50	#10 RV-DRN	5	0
26	42	DRAIN RV	10	0
27	99	END LOOP	1	0
28	56	#10 B RV	12	1
29	2	VORTEX ON	1	0
30	41	VENT RV	1	0
31	88	RS TO RV	1	0
32	93	GAS TO RS	3	0
33	91	#10 TO RS	5	0
34	89	RS TO FC	1	0
35	40	MIX RV	2	0
36	10	GAS B VB	6	0
37	98	BEGIN LOOP	3	0
38	~~20~~ 60	MIX ~~ACT~~ CART	~~1~~ 3	0
39	1	WAIT	2	0
40	99	END LOOP	1	0
41	3	VORTEX OFF	1	0
42	42	DRAIN RV	10	1
43	50	#10 RV-DRN	5	0
44	42	DRAIN RV	15	0
45	9	GAS T VB	3	0
46	10	GAS B VB	3	0
47	93	GAS TO RS	2	0

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	41	VENT RV	1	0
3	88	RS TO RV	1	0
4	93	GAS TO RS	3	0
5	91	#10 TO RS	5	0
6	89	RS TO FC	1	0
7	40	MIX RV	2	0
8	42	DRAIN RV	10	1
9	50	#10 RV-DRN	5	0
10	42	DRAIN RV	10	0
11	98	BEGIN LOOP	4	0
12	56	#10 B RV	13	1
13	2	VORTEX ON	1	0
14	40	MIX RV	3	0
15	10	GAS B VB	6	0
16	60	MIX CART	3	0
17	3	VORTEX OFF	1	0
18	42	DRAIN RV	10	1
19	50	#10 RV-DRN	5	0
20	42	DRAIN RV	10	0
21	99	END LOOP	1	0
22	56	#10 B RV	12	1
23	2	VORTEX ON	1	0
24	41	VENT RV	1	0
25	88	RS TO RV	1	0
26	93	GAS TO RS	3	0
27	91	#10 TO RS	5	0
28	89	RS TO FC	1	0
29	40	MIX RV	2	0
30	10	GAS B VB	6	0
31	98	BEGIN LOOP	3	0
32	60	MIX CART	3	0
33	1	WAIT	2	0
34	99	END LOOP	1	0
35	3	VORTEX OFF	1	0
36	42	DRAIN RV	10	1
37	50	#10 RV-DRN	5	0
38	42	DRAIN RV	15	0
39	9	GAS T VB	3	0
40	10	GAS B VB	3	0
41	93	GAS TO RS	2	0

Module E

1. Copy Std Fmoc mod e to mod E.
2. Delete steps 56 to 73 (18 deletions).
3. Change step 49 to Fxn 62.
4. Delete steps 37 and 38.
5. Delete steps 32 to 34 (3 deletions).
6. Delete steps 3 to 23 (21 deletions).
7. Add the following steps after step 2:

Step#	Fxn#	Function Name	Time	Add
3	10	GAS B VB	3	0
4	98	BEGIN LOOP	5	0
5	44	CART TO RV	6	0
6	2	VORTEX ON	3	0
7	3	VORTEX OFF	2	0
8	99	END LOOP	1	0
9	40	MIX RV	2	0
10	78	PRS #M	10	0
11	70	ML B VB	10	0
12	68	MEAS #7	1	0
13	1	WAIT	1	0
14	43	ML TO RV	1	0
15	10	GAS B VB	5	0
16	65	#10 CART	2	0
17	60	MIX CART	3	0
18	44	CART TO RV	8	0
19	1	WAIT	2	0

Module e:

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	5	NEEDLE DWN	10	0
3	60	MIX CART	2	0
4	10	GAS B VB	3	0
5	20	MIX ACT	1	0
6	2	VORTEX ON	1	0
7	98	BEGIN LOOP	2	0
8	28	GAS T ACT	3	0
9	38	ACT TO RVc	4	0
10	99	END LOOP	1	0
11	3	VORTEX OFF	1	0
12	98	BEGIN LOOP	3	0
13	28	GAS T ACT	2	0
14	38	ACT TO RVc	3	0
15	99	END LOOP	1	0
16	21	VENT ACT	2	0
17	26	#10 T ACT	2	0
18	28	GAS T ACT	3	0
19	21	VENT ACT	2	0
20	36	#10 B ACT	2	0
21	20	MIX ACT	5	0
22	26	GAS T ACT	3	0
23	38	ACT TO RVc	6	0
24	40	MIX RV	2	0
25	93	GAS TO RS	2	0
26	41	VENT RV	2	0
27	88	RS TO RV	1	0
28	93	GAS TO RS	3	0
29	41	VENT RV	2	0
30	89	RS TO FC	1	0
31	2	VORTEX ON	1	0
~~32~~	~~11~~	~~#9 T VB~~	~~1~~	~~0~~
~~33~~	~~21~~	~~VENT ACT~~	~~2~~	~~0~~
~~34~~	~~29~~	~~#9 ACT DRN~~	~~5~~	~~0~~
35	62	DRAIN CART	5	0
36	3	VORTEX OFF	1	0
~~37~~	~~35~~	~~#9 B ACT~~	~~15~~	~~0~~
~~38~~	~~20~~	~~MIX ACT~~	~~2~~	~~0~~
39	67	#10 SML N	2	0
40	66	#9 SML N	2	0
41	62	DRAIN CART	15	0
42	12	#9 B VB	1	0
43	85	BEGIN LOOP	2	0
44	64	#9 CART	20	0
45	2	VORTEX ON	1	0
46	1	WAIT	3	0
47	60	MIX CART	5	0
48	3	VORTEX OFF	1	0
49	~~24~~ 62	~~CART TO AC~~ DRAIN CART	20	0
50	99	END LOOP	1	0
51	2	VORTEX ON	1	0
52	62	DRAIN CART	22	0
53	60	MIX CART	15	0
54	61	VENT CART	5	0
55	3	VORTEX OFF	1	0
56	98	BEGIN LOOP	7	0
57	20	MIX ACT	5	0
58	1	WAIT	5	0
59	95	END LOOP	1	0
60	22	DRAIN ACT	20	0
61	2	VORTEX ON	1	0
62	22	DRAIN ACT	10	0
63	3	VORTEX OFF	1	0
64	98	BEGIN LOOP	3	0
65	29	#9 ACT-DRN	3	0
66	32	DRAIN ACT	5	0
67	99	END LOOP	1	0
68	2	VORTEX ON	1	0
69	12	#9 B VB	1	0
70	35	#9 B ACT	2	0
71	20	MIX ACT	3	0
72	3	VORTEX OFF	1	0
73	22	DRAIN ACT	30	0
74	9	GAS T VB	5	0
75	10	GAS B VB	5	0

Module E (cont.)

Module : E
431A Version : 1.12 Fmoc
Date / Time : 06/08/90 11:44

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	5	NEEDLE DWN	10	0
3	10	GAS B VB	3	0
4	98	BEGIN LOOP	5	0
5	44	CART TO RV	6	0
6	2	VORTEX ON	3	0
7	3	VORTEX OFF	2	0
8	99	END LOOP	1	0
9	40	MIX RV	2	0
10	78	PRS #M	10	0
11	70	ML B VB	10	0
12	68	MEAS #7	1	0
13	1	WAIT	1	0
14	43	ML TO RV	1	0
15	10	GAS B VB	5	0
16	65	#10 CART	2	0
17	60	MIX CART	3	0
18	44	CART TO RV	8	0
19	1	WAIT	2	0
20	40	MIX RV	2	0
21	93	GAS TO RS	2	0
22	41	VENT RV	2	0
23	88	RS TO RV	1	0
24	93	GAS TO RS	3	0
25	41	VENT RV	2	0
26	89	RS TO FC	1	0
27	2	VORTEX ON	1	0
28	62	DRAIN CART	5	0
29	3	VORTEX OFF	1	0
30	67	#10 SML N	2	0
31	66	#9 SML N	2	0
32	62	DRAIN CART	15	0
33	12	#9 B VB	1	0
34	98	BEGIN LOOP	2	0
35	64	#9 CART	20	0
36	2	VORTEX ON	1	0
37	1	WAIT	3	0
38	60	MIX CART	5	0
39	3	VORTEX OFF	1	0
40	62	DRAIN CART	20	0
41	99	END LOOP	1	0
42	2	VORTEX ON	1	0
43	62	DRAIN CART	10	0
44	60	MIX CART	15	0
45	61	VENT CART	5	0
46	3	VORTEX OFF	1	0
47	9	GAS T VB	5	0
48	10	GAS B VB	5	0

Module F

1. Copy Std Fmoc mod f to mod F.
2. Delete steps 20 and 21.
3. On step 18, change the loop from 18 to 25.
4. Add the following after step 10:

Fxn#	Function Name	Time	Add
14	#10 B VB	2	0
56	#10 B RV	1	0
40	MIX RV	3	0

1. Delete steps 7 and 8.
2. On step 5, change the loop from 18 to 25.

Module f:

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	13	#10 T VB	1	0
3	10	GAS B VB	5	0
4	9	GAS T VB	5	0
5	98	BEGIN LOOP	~~18~~ 25	0
6	2	VORTEX ON	13	0
~~7~~	~~10~~	~~GAS B VB~~	~~3~~	~~0~~
~~8~~	~~20~~	~~MIX ACT~~	~~1~~	~~0~~
9	3	VORTEX OFF	15	0
10	99	END LOOP	1	0
11	91	#10 TO RS	1	0
12	93	GAS TO RS	2	0
13	41	VENT RV	2	0
14	88	RS TO RV	1	0
15	93	GAS TO RS	2	0
16	41	VENT RV	1	0
17	89	RS TO FC	1	0
18	98	BEGIN LOOP	~~18~~ 25	0
19	2	VORTEX ON	13	0
~~20~~	~~10~~	~~GAS B VB~~	~~3~~	~~0~~
~~21~~	~~20~~	~~MIX ACT~~	~~1~~	~~0~~
22	3	VORTEX OFF	15	0
23	99	END LOOP	1	0
24	9	GAS T VB	2	0
25	13	#10 T VB	1	0
26	9	GAS T VB	5	0
27	14	#10 B VB	1	0
28	10	GAS B VB	5	0

Module : F

431A Version : 1.12 Fmoc

Date / Time : 06/08/90 11:45

Step#	Fxn#	Function Name	Time	Add
1	14	#10 B VB	1	0
2	13	#10 T VB	1	0
3	10	GAS B VB	5	0
4	9	GAS T VB	5	0
5	98	BEGIN LOOP	25	0
6	2	VORTEX ON	13	0
7	3	VORTEX OFF	15	0
8	99	END LOOP	1	0
9	14	#10 B VB	2	0
10	56	#10 B RV	1	0
11	40	MIX RV	3	0
12	91	#10 TO RS	1	0
13	93	GAS TO RS	2	0
14	41	VENT RV	2	0
15	88	RS TO RV	1	0
16	93	GAS TO RS	2	0
17	41	VENT RV	1	0
18	89	RS TO FC	1	0
19	98	BEGIN LOOP	25	0
20	2	VORTEX ON	13	0
21	3	VORTEX OFF	15	0
22	99	END LOOP	1	0
23	9	GAS T VB	2	0
24	13	#10 T VB	1	0
25	9	GAS T VB	5	0
26	14	#10 B VB	1	0
27	10	GAS B VB	5	0

Module G

1. Copy Std Fmoc mod g to mod G.
2. On step 30, change the loop from 4 to 2.
3. Delete steps 8 to 17 (10 deletions).

Module g:

Step#	Fxn#	Function Name	Time	Add
1	13	#10 T VB	1	0
2	91	#10 TO RS	2	0
3	41	VENT RV	2	0
4	88	RS TO RV	1	0
5	93	GAS TO RS	3	0
6	91	#10 TO RS	5	0
7	89	RS TO FC	1	0
8	98	BEGIN LOOP	3	0
9	14	#10 B VB	1	0
10	40	MIX RV	4	0
11	99	END LOOP	1	0
12	2	VORTEX ON	5	0
13	40	MIX RV	4	0
14	3	VORTEX OFF	1	0
15	40	MIX RV	4	0
16	10	GAS B VB	6	0
17	20	MIX ACT	1	0
18	42	DRAIN RV	20	2
19	56	#10 B RV	14	1
20	41	VENT RV	1	0
21	88	RS TO RV	1	0
22	91	#10 TO RS	5	0
23	89	RS TO FC	1	0
24	2	VORTEX ON	3	0
25	40	MIX RV	3	0
26	3	VORTEX OFF	1	0
27	42	DRAIN RV	15	1
28	50	#10 RV-DRN	4	0
29	42	DRAIN RV	10	0
30	98	BEGIN LOOP	~~4~~ 2	0
31	56	#10 B RV	14	1
32	2	VORTEX ON	1	0
33	40	MIX RV	3	0
34	3	VORTEX OFF	1	0
35	42	DRAIN RV	10	1
36	50	#10 RV-DRN	4	0
37	42	DRAIN RV	10	0
38	99	END LOOP	1	0
39	56	#10 B RV	14	1
40	41	VENT RV	1	0
41	88	RS TO RV	1	0
42	91	#10 TO RS	5	0
43	89	RS TO FC	1	0
44	2	VORTEX ON	1	0
45	40	MIX RV	3	0
46	10	GAS B VB	6	0
47	20	MIX ACT	1	0
48	3	VORTEX OFF	1	0
49	42	DRAIN RV	15	1
50	14	#10 B VB	1	0
51	56	#10 B RV	14	1
52	2	VORTEX ON	1	0
53	40	MIX RV	1	0
54	91	#10 TO RS	2	0
55	93	GAS TO RS	2	0
56	90	#9 TO RS	2	0
57	93	GAS TO RS	1	0
58	91	#10 TO RS	2	0
59	93	GAS TO RS	2	0
60	98	BEGIN LOOP	1	0
61	39	RELAY 0	1	0
62	13	#10 T VB	1	0
63	41	VENT RV	2	0
64	88	RS TO RV	1	0
65	91	#10 TO RS	2	0
66	93	GAS TO RS	1	0
67	91	#10 TO RS	2	0
68	41	VENT RV	1	0
69	48	GAS T RV	1	0
70	87	TAKE SAMPL	2	0
71	89	RS TO FC	1	0
72	90	#9 TO RS	2	0
73	93	GAS TO RS	2	0
74	90	#9 TO RS	2	0
75	93	GAS TO RS	2	0
76	90	#9 TO RS	4	0
77	93	GAS TO RS	4	0
78	41	VENT RV	2	0
79	88	RS TO RV	1	0
80	91	#10 TO RS	3	0
81	93	GAS TO RS	3	0
82	91	#10 TO RS	5	0
83	89	RS TO FC	1	0
84	39	RELAY 0	1	0
85	99	END LOOP	1	0
86	3	VORTEX OFF	1	0
87	42	DRAIN RV	20	2

Module G (cont.)

Module : G
431A Version : 1.12 Fmoc
Date / Time : 06/08/90 11:45

Step#	Fxn#	Function Name	Time	Add
1	13	#10 T VB	1	0
2	91	#10 TO RS	2	0
3	41	VENT RV	2	0
4	88	RS TO RV	1	0
5	93	GAS TO RS	3	0
6	91	#10 TO RS	5	0
7	89	RS TO FC	1	0
8	42	DRAIN RV	20	2
9	56	#10 B RV	14	1
10	41	VENT RV	1	0
11	88	RS TO RV	1	0
12	91	#10 TO RS	5	0
13	89	RS TO FC	1	0
14	2	VORTEX ON	3	0
15	40	MIX RV	3	0
16	3	VORTEX OFF	1	0
17	42	DRAIN RV	15	1
18	50	#10 RV-DRN	4	0
19	42	DRAIN RV	10	0
20	98	BEGIN LOOP	2	0
21	56	#10 B RV	14	1
22	2	VORTEX ON	1	0
23	40	MIX RV	3	0
24	3	VORTEX OFF	1	0
25	42	DRAIN RV	10	1
26	50	#10 RV-DRN	4	0
27	42	DRAIN RV	10	0
28	99	END LOOP	1	0
29	56	#10 B RV	14	1
30	41	VENT RV	1	0
31	88	RS TO RV	1	0
32	91	#10 TO RS	5	0
33	89	RS TO FC	1	0
34	2	VORTEX ON	1	0
35	40	MIX RV	3	0
36	10	GAS B VB	6	0
37	20	MIX ACT	1	0
38	3	VORTEX OFF	1	0
39	42	DRAIN RV	15	1
40	14	#10 B VB	1	0

Step#	Fxn#	Function Name	Time	Add
41	56	#10 B RV	14	1
42	2	VORTEX ON	1	0
43	40	MIX RV	1	0
44	91	#10 TO RS	2	0
45	93	GAS TO RS	2	0
46	90	#9 TO RS	2	0
47	93	GAS TO RS	1	0
48	91	#10 TO RS	2	0
49	93	GAS TO RS	2	0
50	98	BEGIN LOOP	1	0
51	39	RELAY 0	1	0
52	13	#10 T VB	1	0
53	41	VENT RV	2	0
54	88	RS TO RV	1	0
55	91	#10 TO RS	2	0
56	93	GAS TO RS	1	0
57	91	#10 TO RS	2	0
58	41	VENT RV	1	0
59	48	GAS T RV	1	0
60	87	TAKE SAMPL	2	0
61	89	RS TO FC	1	0
62	90	#9 TO RS	2	0
63	93	GAS TO RS	2	0
64	90	#9 TO RS	2	0
65	93	GAS TO RS	2	0
66	90	#9 TO RS	4	0
67	93	GAS TO RS	4	0
68	41	VENT RV	2	0
69	88	RS TO RV	1	0
70	91	#10 TO RS	3	0
71	93	GAS TO RS	3	0
72	91	#10 TO RS	5	0
73	89	RS TO FC	1	0
74	39	RELAY 0	1	0
75	99	END LOOP	1	0
76	3	VORTEX OFF	1	0
77	42	DRAIN RV	20	2

Small Scale (0.10 mmol)

At 20 minutes per cycle, the 0.10 mmol cycles are extremely fast. In our laboratory we have successfully synthesized peptides of up to 21 amino acids in length. For longer peptides, you may need to extend the 7 minute deprotection and 9 minute coupling times.

Note The amino acid cartridge disposal bucket on the Model 431A holds approximately 25 used cartridges. With the faster *FastMoc*™ cycles, this bucket quickly becomes filled to overflowing when you synthesize peptides longer than 25 amino acids. Remove the bucket altogether when making longer peptides to prevent an accumulation of discarded cartridges that can back up and interfere with the cartridge ejection mechanism.

FastMoc™ cycles are usually used with the non-resin sampling small scale reaction vessel (refer to Chapter 1, Synthesis Setup, of the Model 431A Users Manual). The Fmoc-amino acid is dissolved and activated in the cartridge in a mixture of 2.2 g of 0.45 M HBTU/HOBt in DMF, 0.22 g (1.7 eq.) DIEA, and 0.8 mL NMP.

Note If you wish to take resin samples, you need to use a small scale resin-sampling reaction vessel (P/N 400976).

FastMoc™ Module Descriptions (0.10 mmol)

Module A: Dissolution of amino acid **Total time = 5.3 min**

The amino acid cartridge name is printed, the old cartridge is ejected and the new one is advanced. After reagent manifold pressurization, 2.2 g of 0.45 M HBTU/HOBt in DMF is delivered to the cartridge, followed by 0.8 mL of NMP and 0.22 g of DIEA. The amino acid is dissolved during 4.4 minutes of mixing while the reaction vessel is intermittently vortexed.

Module B: Piperidine delivery **Total time = 2.0 min**

After draining, 1.0 mL of piperidine and 1.0 mL NMP are delivered to the reaction vessel for a one-minute treatment. The reaction vessel is drained and 1.0 mL of piperidine and 1.0 mL NMP are added again. The valve blocks are rinsed with NMP and gas dried. There are about 1.5 minutes of deprotection time during this module.

Module D: NMP RV washes **Total time = 1.7 min**

After draining, the resin is washed with NMP three times. The cartridge is mixed intermittently for 5 seconds at a time. This module is used between deprotection and coupling.

Module E: Transfer and needle washing **Total time = 3.8 min**

The activated amino acid solution is transferred to the reaction vessel and the cartridge and needles are washed several times with NMP. The washing steps allow for 3.2 minutes of coupling time during this module.

Module F: RV vortexing **Total time = 5.2 min**

The reaction vessel is vortexed in 15-second intervals for a total time of 5.2 minutes. This module consists of a single half-minute loop that is executed ten times. To extend the deprotection and/or coupling time, you may insert extra F modules in the Run Editor.

FastMoc™ Modules for the Run Editor (0.10 mmol)

For every amino acid to be coupled, use the following series of modules for a synthesis without resin sampling:

DBADEF

If pre-loaded resin or C-terminal amide resins are used, use this cycle for the first and for subsequent cycles.

If you are loading the C-terminal amino acid to an HMP resin, use the following modules for the first cycle:

heffffffffg

If you are loading the C-terminal amino acid to an HMP resin and capping, use the following modules for the first cycle:

heffffffffgheffffffffg

The first cartridge is the amino acid, followed by a cartridge containing 2.0 mmol (0.45 g) benzoic anhydride.

If you are loading His or Arg, consult the Model 431A Users Manual, Chapter 5, Loading Modifications section. For all subsequent cycles use:

DBADEF

If you want the final Fmoc group to be removed, use the following modules for the final cycle:

DBFDc

For peptides longer than 20 amino acids, extend the coupling and deprotection times with the following modules:

DBFADEFF

For a double couple, use the following modules:

DBFADEFADEFF

Use two amino acid cartridges for a double couple.

If you want to take resin samples, place module g at the end of each coupling cycle.

regular cycle:	DBADEFg
longer cycle:	DBFADEFFg
double couple cycle:	DBFADEFADEFFg

Examples for Run Editor (0.10 mmol)

Example 1: Angiotensin, a 10-mer peptide

To synthesize Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu on HMP resin with removal of the final Fmoc group but without resin sampling:

Cy: 1	Rpt: 1	M: heffffffg
Cy: 2	Rpt: 9	M: DBADEF
Cy: 11	Rpt: 1	M: DBFDc

Example 2: Angiotensin, a 10-mer peptide

To synthesize Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu on HMP resin with capping after loading and removal of final Fmoc group, but without resin sampling:

Cy: 1	Rpt: 1	M: heffffffgheffffffg
Cy: 2	Rpt: 9	M: DBADEF
Cy: 11	Rpt: 1	M: DBFDc

Example 3: Angiotensin, a 10-mer peptide

To synthesize angiotensin on Fmoc-Leu-resin with removal of the final Fmoc group but without resin sampling:

Cy: 1	Rpt: 9	M: DBADEF
Cy: 10	Rpt: 1	M: DBFDc

Example 4: Angiotensin, a 10-mer peptide

To synthesize angiotensin on Fmoc-Leu-resin, without removal of the final Fmoc group and without resin sampling:

Cy: 1	Rpt: 9	M: DBADEF
Cy: 10	Rpt: 1	M: c

Example 5: Substance P, an 11-mer amide peptide

To synthesize Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2 on Rink resin with removal of the final Fmoc groups but without resin sampling:

Cy: 1	Rpt: 11	M: DBADEF
Cy: 12	Rpt: 1	M: DBFDc

0.10 mmol *FastMoc™* Modules

To use modules c, e, f, g, and h, the Model 431A must be set on the small scale Fmoc (SSFmoc) cycles.

Module A

Step #	Fxn #	Function Name	Time	Add
1	4	PRINT CART	10	0
2	6	NEEDLE UP	10	0
3	7	EJECT CART	10	0
4	8	ADVAN CART	10	0
5	5	NEEDLE DWN	10	0
6	78	PRS #M	10	0
7*	**94**	**USER FXN A**	**9****	0
8	65	#10 CART	2	0
9	60	MIX CART	5	0
10*	95	USER FXN B	1	0
11*	97	USER FXN D	1	0
12*	95	USER FXN B	1	0
13*	97	USER FXN D	1	0
14	63	ML TO CART	1	0
15	70	ML B VB	5	0
16	98	BEGIN LOOP	8	0
17	2	VORTEX ON	1	0
18	60	MIX CART	15	0
19	3	VORTEX OFF	1	0
20	60	MIX CART	15	0
21	99	END LOOP	1	0

* Steps 7, 9, 10, 11 and 12 are User Functions that activate the following valves:

Fxn 94:	User Fxn A	Valves:	12, 15, 27, 30
Fxn 95:	User Fxn B	Valves:	6, 13
Fxn 97:	User Fxn D	Valves:	30, 31

** The value for the time in step 7 is determined by running module H, (described on page 6) and it varies slightly from instrument to instrument. We have used either 8, 9, or 10 seconds in our developmental work. The objective is to have 2.1 to 2.3 g of the 0.45 M HBTU solution delivered to the cartridge.

Module B

Step #	Fxn #	Function Name	Time	Add
1	42	DRAIN RV	10	1
2	79	PRS #1	10	0
3	51	#1 B RV	5	0
4	56	#10 B RV	2	0
5	40	MIX RV	2	0
6	98	BEGIN LOOP	2	0
7	2	VORTEX ON	15	0
8	3	VORTEX OFF	15	0
9	99	END LOOP	1	0
10	79	PRS #1	10	0
11	42	DRAIN RV	10	1
12	51	#1 B RV	5	0
13	56	#10 B RV	2	0
14	40	MIX RV	2	0
15	98	BEGIN LOOP	2	0
16	2	VORTEX ON	1	0
17	13	#10 T VB	3	0
18	14	#10 B VB	3	0
19	3	VORTEX OFF	1	0
20	9	GAS T VB	3	0
21	10	GAS B VB	3	0
22	99	END LOOP	1	0

Module D

Step #	Fxn #	Function Name	Time	Add
1	5	NEEDLE DWN	10	0
2	98	BEGIN LOOP	3	0
3	50	#10 RV-DRN	2	0
4	42	DRAIN RV	10	1
5	56	#10 B RV	4	1
6	88	RS TO RV	1	0
7	91	#10 TO RS	3	0
8	89	RS TO FC	1	0
9	40	MIX RV	2	0
10	2	VORTEX ON	1	0
11	9	GAS T VB	2	0
12	10	GAS B VB	2	0
13	60	MIX CART	5	0
14	3	VORTEX OFF	1	0
15	99	END LOOP	1	0

Module E

Step #	Fxn #	Function Name	Time	Add
1	5	NEEDLE DWN	10	0
2	42	DRAIN RV	10	1
3	9	GAS T VB	3	0
4	10	GAS B VB	3	0
5	98	BEGIN LOOP	3	0
6	41	VENT RV	3	0
7*	96	USER FXN C	10	0
8	99	END LOOP	1	0
9	40	MIX RV	1	0
10	98	BEGIN LOOP	3	0
11	2	VORTEX ON	1	0
12	67	#10 SML N	5	0
13	3	VORTEX OFF	1	0
14	65	#10 CART	10	0
15	41	VENT RV	2	0
16	60	MIX CART	10	0
17	62	DRAIN CART	20	0
18	9	GAS T VB	3	0
19	10	GAS B VB	3	0
20	99	END LOOP	1	0

* Step 7 is a User Function that activates the following valves: 10, 12, 17, and 22.

Module F

Step #	Fxn #	Function Name	Time	Add
1	98	BEGIN LOOP	10	0
2	2	VORTEX ON	15	0
3	3	VORTEX OFF	13	0
4	41	VENT RV	2	0
5	99	END LOOP	1	0

References

1. Knorr, R., et al., 1989. Tetrahedron Letters, 30:1927-1930.
2. Breipohl, G., personal communication
3. Fields, C., et al., Applied Biosystems Research News (in press).

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