

PROCEDURE FOR THE SYNTHESIS, DEPROTECTION AND ISOLATION OF OLIGONUCLEOSIDE PHOSPHORODITHIOATE LINKAGE USING SULFUR-LINKED BASE-LABILE PROTECTED MONOMERS

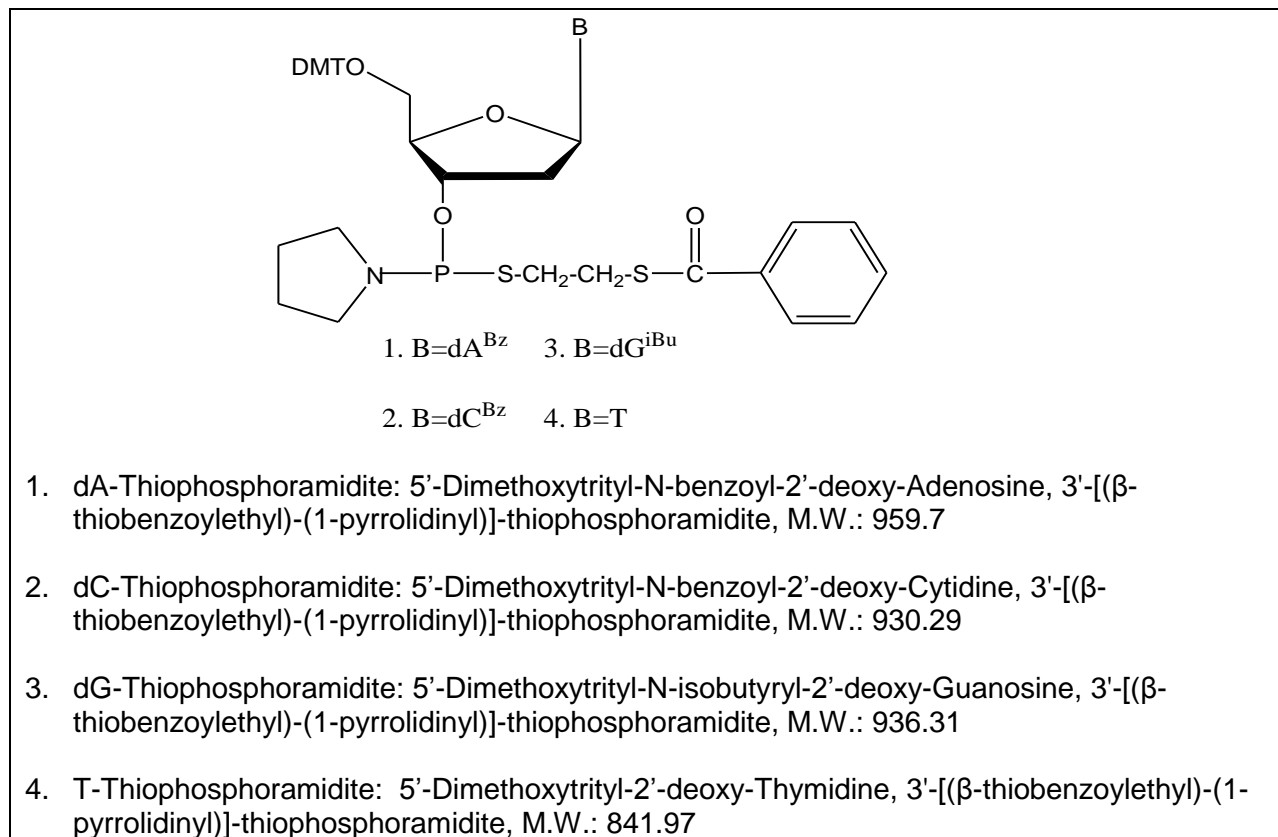


Figure 1: Structure of Thiophosphoramidite Monomers

Introduction

2'-Deoxyoligonucleoside phosphorodithioate (PS2-ODN) synthesis using thiophosphoramidite monomers (**Fig. 1**) is characterized by very high coupling efficiencies along with industry standard chemical DNA synthesis and deprotection which results in a nucleic acid analog that contains achiral internucleotide phosphodiester groups with two sulfurs substituting for the two nonbridging oxygen atoms resulting in a phosphorodithioate linkage. Initially, PS2-ODNs were investigated as potential antisense compounds and exhibited the ability to interfere with the expression of erbB-2 mRNA associated with breast cancer, to inhibit HIV-1 reverse transcription activity and to induce B-cell proliferation and differentiation. The PS2-ODN analogues have demonstrated their utility as potent inhibitors of HIV-1 reverse transcriptase and of HIV-1 viral replication. Oligodeoxycytidine PS2 analogs exhibit strong inhibitory properties

against HIV-1 reverse transcriptase, a viral enzyme which is essential for HIV replication, as well as HIV-1 replication. This inhibition is dependent on both the number of dithioate linkages and the length of dithioate oligomer analog. A comparative analysis with phosphoromonothioate equivalents indicates that dithioates are much better inhibitors and are able to inhibit potently with relatively short oligomer length. Inhibition of HIV-1 reverse transcriptase by PS2-ODN appears to be a general phenomenon as all of the nucleotide base sequences examined inhibits its activity. In addition the PS2 analogues have been used successfully as aptamers for a variety of protein targets, including transcription factor NF- κ B and activated-protein 1 AP-1 with nM or sub-nM Kds.

Preparation of thiophosphoramidite monomers solution

Thiophosphoramidite monomers can not be purified via the column chromatography procedures that are used for the industry standard 2'-deoxynucleoside 3'-phosphoramidites. Using both patented thiophosphoramidite synthesis procedures (US Patent No. 5218088) and published literature techniques AM recently developed a repeatable large scale process for making the patented thiophosphoramidites in excellent yields (85% at greater than 90% purity by ^{31}P -NMR attached).

Diluents: Acetonitrile, anhydrous (AM_diluent A, alternatively, Glen Res. 40-4050-XX)
Dichloromethane, anhydrous (AM_diluent B, alternatively, Aldrich 270997)

Concentrations: 0.05 M or higher for Expedite 8909 DNA Synthesizer have been successfully used in our lab for many years. This is the lowest concentration for Expedite 8909. Higher concentrations or optimized concentrations may be required for other instruments. Be aware that **0.15 M solutions and double couplings were used for most of the published articles.**

dA-Thiophosphoramidite:	0.05 M solution in anhydrous acetonitrile containing ~ 12% dichloromethane
dC-Thiophosphoramidite:	0.05 M solution in anhydrous acetonitrile containing ~ 3% dichloromethane
dG-Thiophosphoramidite:	0.05 M solution in anhydrous acetonitrile containing ~ 13% dichloromethane
T-Thiophosphoramidite:	0.05 M solution in anhydrous acetonitrile containing ~ 2% dichloromethane

Note: It is strongly recommended that 3 Å molecular sieves (Aldrich 20858-2, dried in an oven at ~ 200 °C for 20 hrs, cooling to room temperature under vacuum and exposed to argon) should be added to the 0.05 M thiophosphoramidite solution.

Phosphorodithioate oligonucleotide synthesis

The solid-phase synthesis of phosphorodithioate DNA oligomers is similar to conventional procedures for preparing deoxyoligonucleotides, *but there are a few important modifications.* Following detritylation of a 2'-deoxynucleoside linked to controlled pore glass,

the 2'-deoxynucleoside 3'-thiophosphoramidite synthons are coupled to the growing oligomers on the support by conventional tetrazole activation (Glen Res. 30-3102-XX). The resulting thiophosphite triester is oxidized with Beaucage's reagent. Unreacted support-linked 2'-deoxynucleoside is then capped, which completes the cycle for addition of nucleotide.

Protocol for Expedite 8909 DNA synthesizer is attached. **PLEASE NOTE THAT THE PROCEDURE IS NOT RECOMMENDED BY THE INSTRUMENT MANUFACTURER.** The coupling time in the protocol provided by the manufacturer is not efficient! Coupling times of more than 3 minute (double couplings) have been used successfully in the past several years. Tetrazole has also been used successfully as activator. The time for the sulfurization step is about 4 minutes.

If you synthesize a mixed normal phosphate and dithiophosphate backbone DNA, you have to modify the oxidation procedure recommended by the instrument manufacturer because the oxidation time in the protocol provided by the manufacturer is not efficient. You may follow our attached protocol. Be aware that if the oxidation step is not complete at this step, you will create phosphoromonothioate DNA in the following sulfurization step for creating phosphorodithioate linkages!

Cleavage from the support and remove of base and phosphate protecting groups

On completion of the automated synthesis, the CPG column is dried with argon. The CPG support (1 μ mole scale) was treated with 0.5 mL of concentrated ammonia (>28%) for 1 h at room

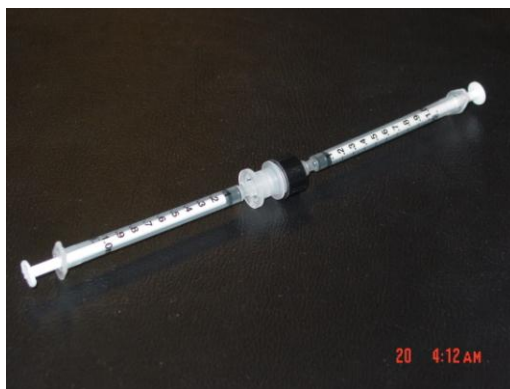


Figure 2: Setup for Treating the CPG Support

temperature (see **Fig. 2**). Afterwards, the support is treated with an additional 0.5 mL of concentrated ammonia for 0.5 h at room temperature. The collected solution is sealed and incubated at 55 °C for 15-16 h. After the vial is removed from the oven it is cooled to room temperature. The solution is transferred to a large 15 ml vial and distilled water is added to make 5 mL of total solution. The solvents are removed by lyophilization.

Purifying the PS2-ODN

Synthetic PS2-ODNs have been purified by PAGE, AEC and RP-HPLC. While PAGE allows for excellent separation of PS2-ODNs with greater than 97% purity in most cases, the technique suffers from numerous complications including the necessity of manual band

excision. In addition, gel loading capacity is typically low and limits the usefulness of PAGE as a preparative method. RP-HPLC is commonly used with the “DMT-on” method. This group is highly hydrophobic and the full length product with the DMT group is highly retained on the reversed-phase column. The purification is relatively quick and offers purities ~ 95%; however, the DMT group must be removed by acid hydrolysis after purification. Despite its many advantages for normal ODN isolation, RP-HPLC is not the method of choice to purify PS2-ODNs. The very complex chromatogram of a crude synthetic mixture of PS2-ODN on a reversed-phase column makes its use for purification of PS2-ODNs undesirable. AEC-MQC separates PS2-ODNs based on the number of charges and sulfurs and is very efficient for lengths from 2- to 30-mers with purity around 95%. High quality purified PS2-ODNs with less than 50% PS2 linkages have been obtained and reported by our laboratory with this method. In any purification system for PS2-ODNs this AEC-MQC step is necessary to separate full-length, dithioated products from failure, monothioated, and desulfurization products. We have previously reported that the retention times on the AEC-MQC of PS2-ODNs containing different numbers of PS2 linkages under the same gradient conditions were found to be a linear function of the number of sulfurs, not of the sequence composition of the PS2-ODNs. This linear relationship is further supported when we compare the retention times of two 5'-thiophosphate 14-mer sequences and their 5'-phosphate sequences. The average retention time increase (2.33 min per sulfur, Mono-Q 5/5).

Mono Q Ion-exchange Chromatography

High-performance ion-exchange chromatography was performed with the Dynamax analytical HPLC system from Varian. The system consists of two Dynamax SD-200 pumps and Dynamax UV-D II dual-wavelength programmable detector, and a 9725 Rheodyne injector with a 2 ml sample loop with Macintosh-based HPLC control and data acquisition. The columns used were Mono Q HR 5/5 (flow rate 1.0 ml/min) and 10/10 (flow rate 2.0 ml/min). The buffers used were (A) 25 mM Tris-HCl, 1 mM EDTA, pH 8 and (B) 25 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8. Separation of the DNA species was achieved by gradient elution, and the gradient profile used is summarized in Table 1. The UV monitor was set at 254 nm for gradients.

Alternate AEC-MQC Instrumentation and Conditions

The crude synthetic PS2-ODN separations can be performed on an AKTA FPLC P-920 (Amersham Biosciences) system controlled by their Unicorn software. The column used is a Mono Q 5/5 (Amersham Biosciences). The buffers used were (A) 1 mM EDTA, 25 mM Tris-HCl, pH 8; (B) 1 mM EDTA, 25 mM Tris-HCl, 1 M NaCl, pH 8. Separation of the PS2-ODNs is achieved by gradient elution and the gradient profiles used is as follows: 0-100% B in 80 minutes, hold at 100% B for 4 minutes, 100% B to 0% B in 1 minute, hold at 0% B for 5 minutes. The UV monitor is set at 254 nm. The flow rate was 1 ml/min.

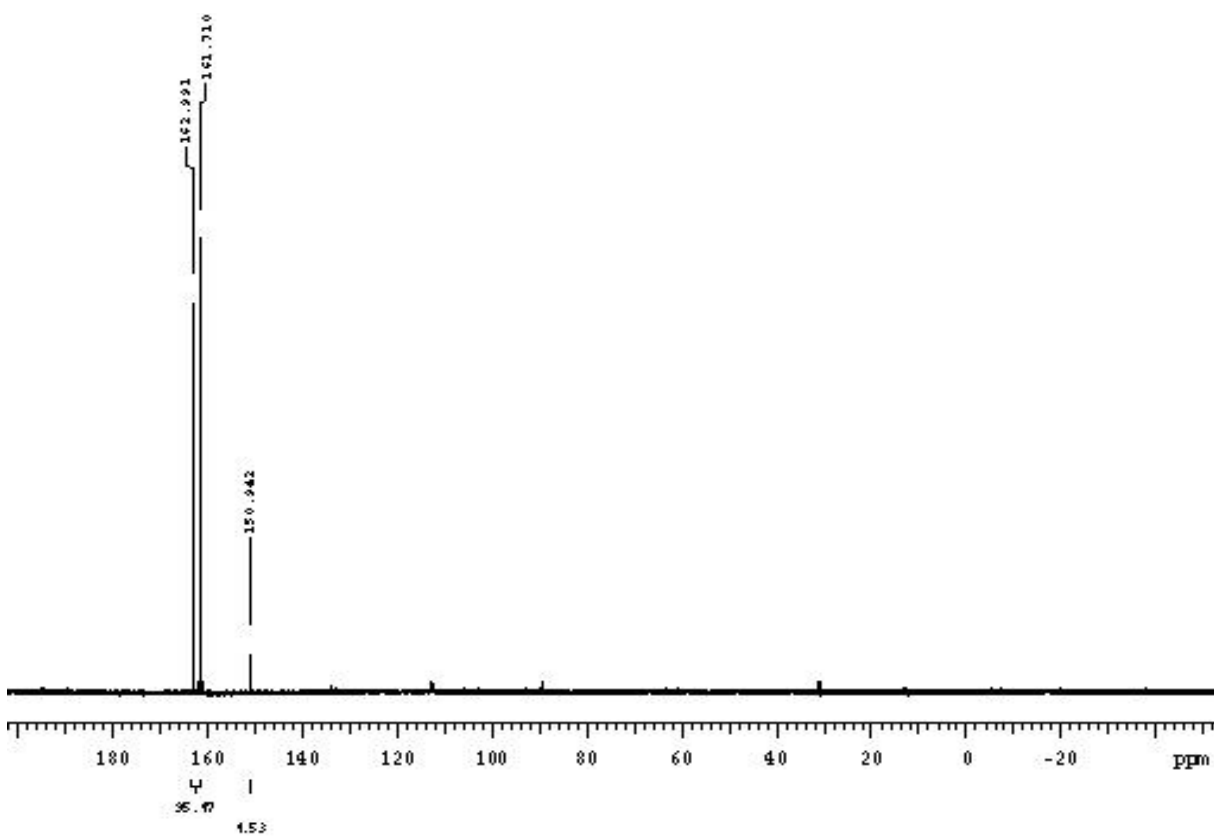
Characterization of PS2-ODN

PS2-ODN can be finally confirmed by MALDI-TOF MS and ³¹P-NMR. Resonances of PS2 linkage (~ 112 ppm), PS linkage (~ 58 ppm) and phosphate (PO) (~ 0 ppm) in ³¹P-NMR spectra are well resolved. Thus the contaminated PS-ODN impurities were assayed by ³¹P NMR.

Attachments

(1) dA-Thiophosphoramidite (Lot#: AM_32):

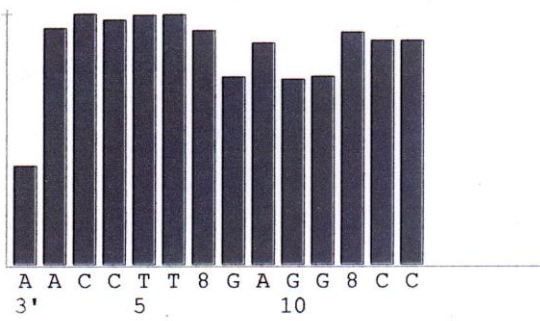
^{31}P -NMR (161MHz) in CD_2Cl_2



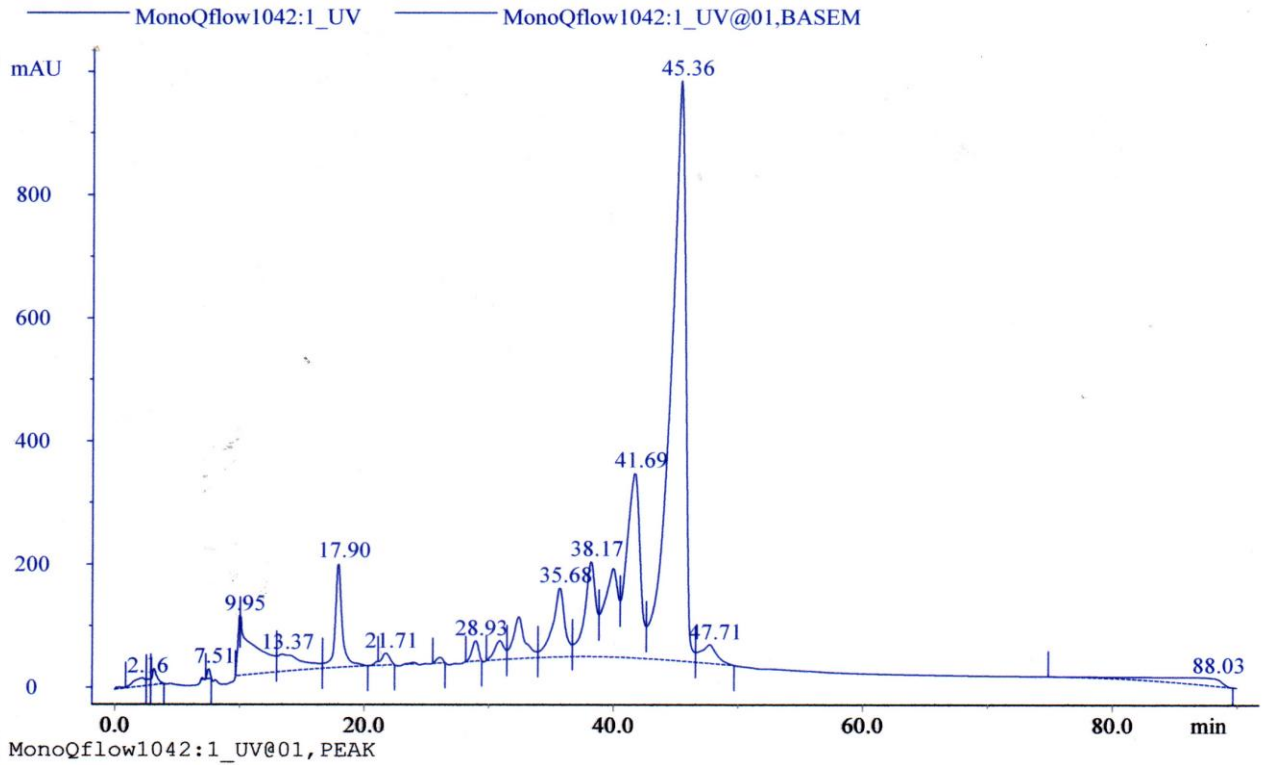
Testing sequence (AA_15): 5'-CCA_{S2}GGAGA_{S2}TTCAA-3'

(a) Synthesis scale: 1 μ mole, C=0.05 M

DMT data:

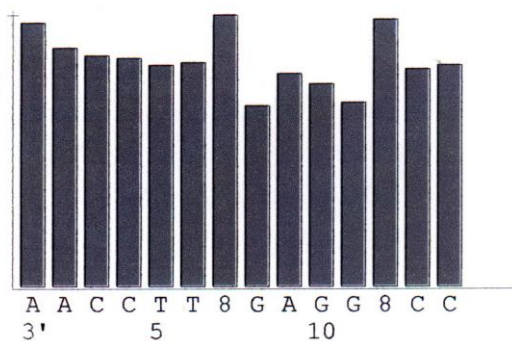


FPLC chromatographic of the crude synthesis

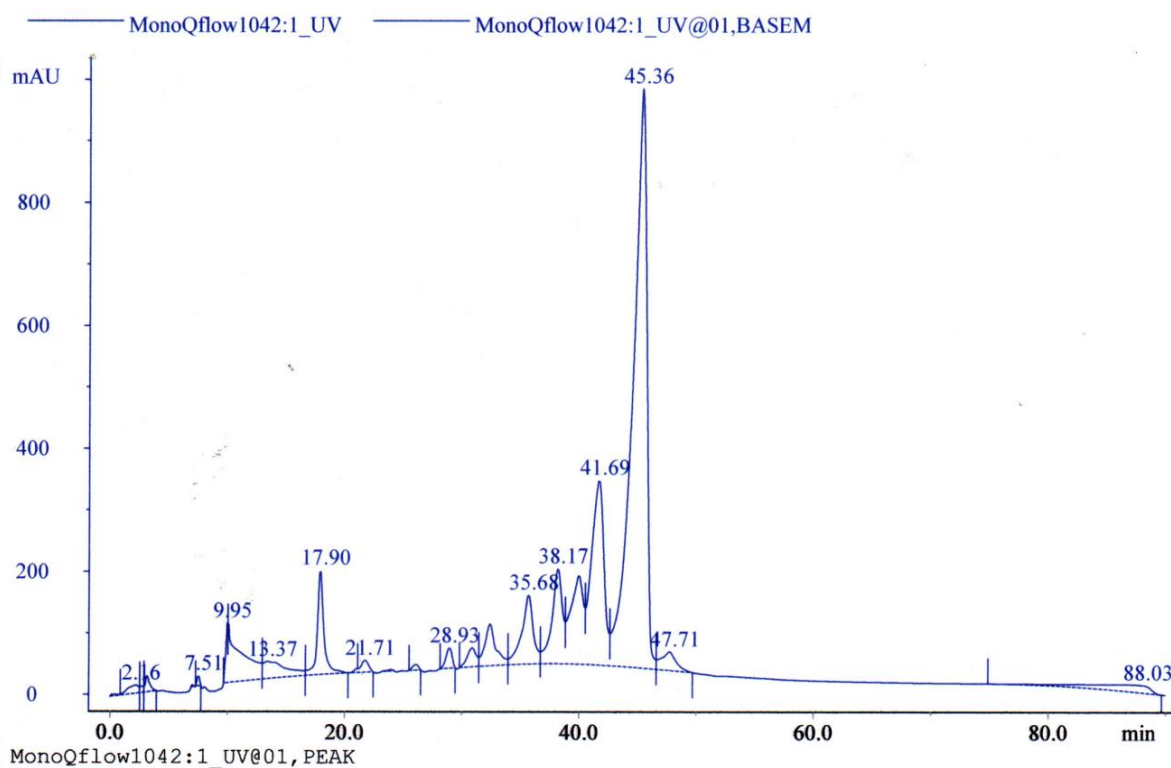


(b) Synthesis scale: 0.2 μ mole, C=0.05 M

DMT data:

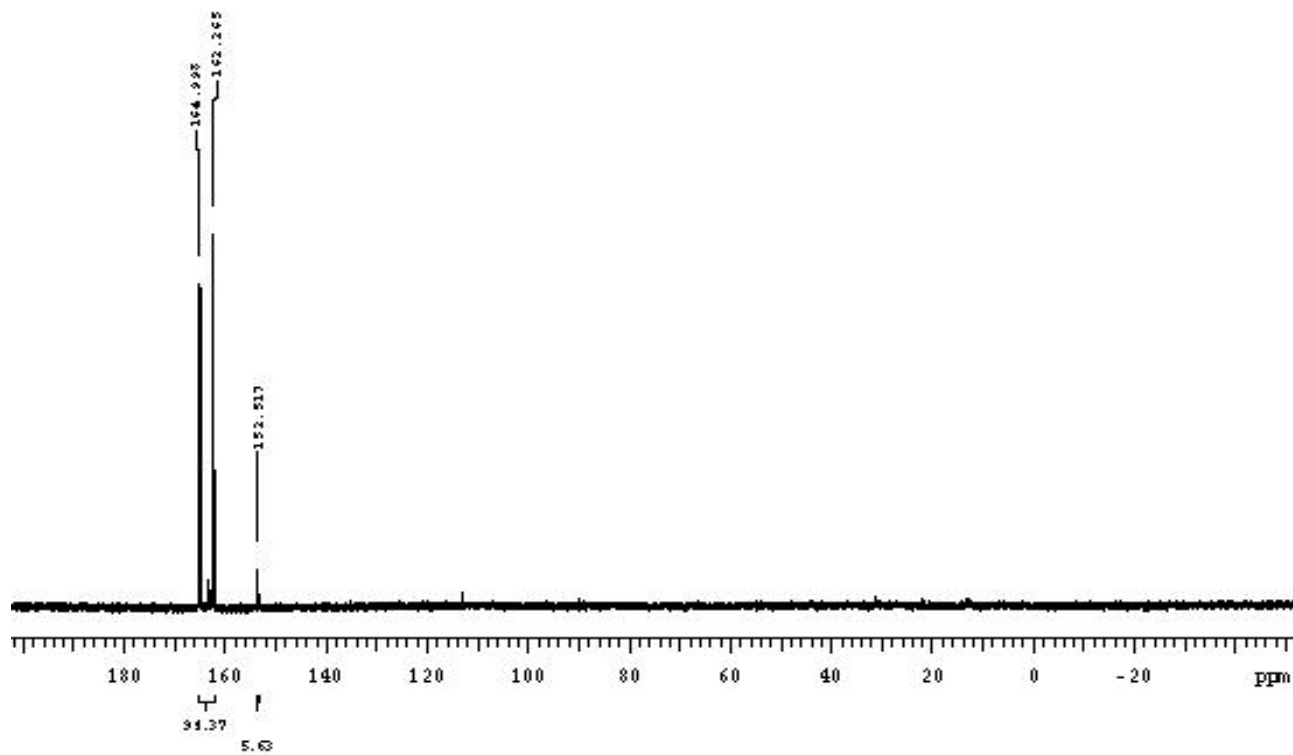


FPLC chromatographic of the crude synthesis



(2) dC-Thiophosphoramidite (lot#: AM_33):

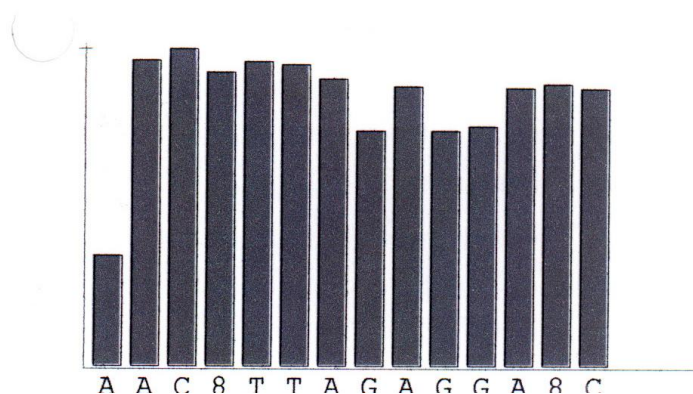
^{31}P -NMR (161MHz) in CD_2Cl_2



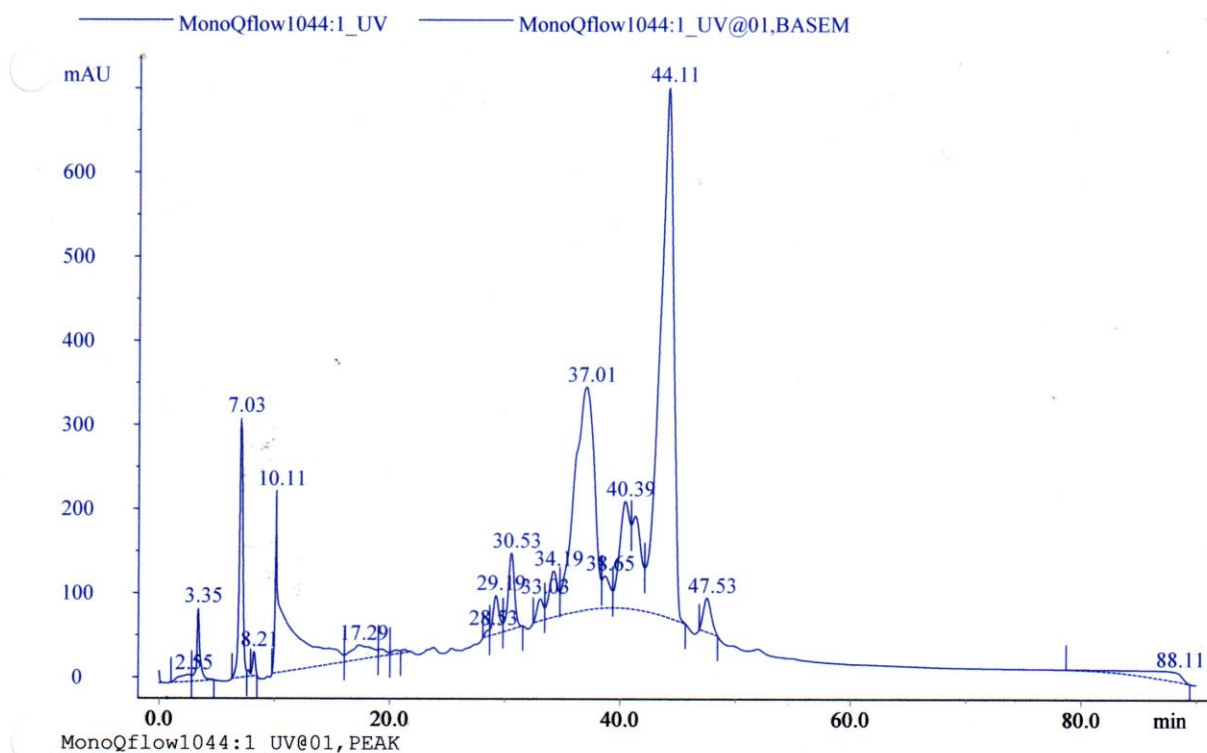
Testing sequence (AA_17): 5'-CC_{S2}AGGAGATTC_{S2}CAA-3'

(a) Synthesis scale: 1 μ mole, C=0.05 M

DMT data:

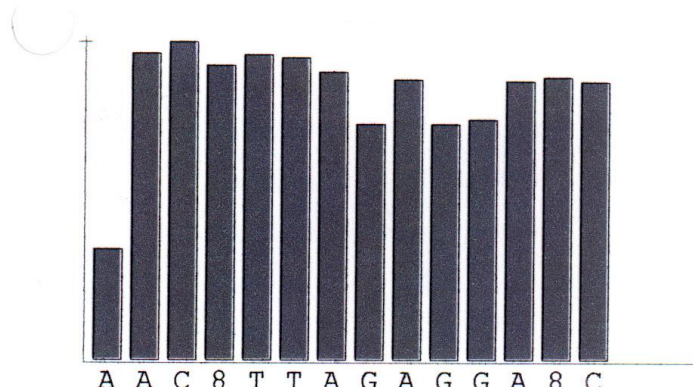


FPLC chromatographic of the crude synthesis

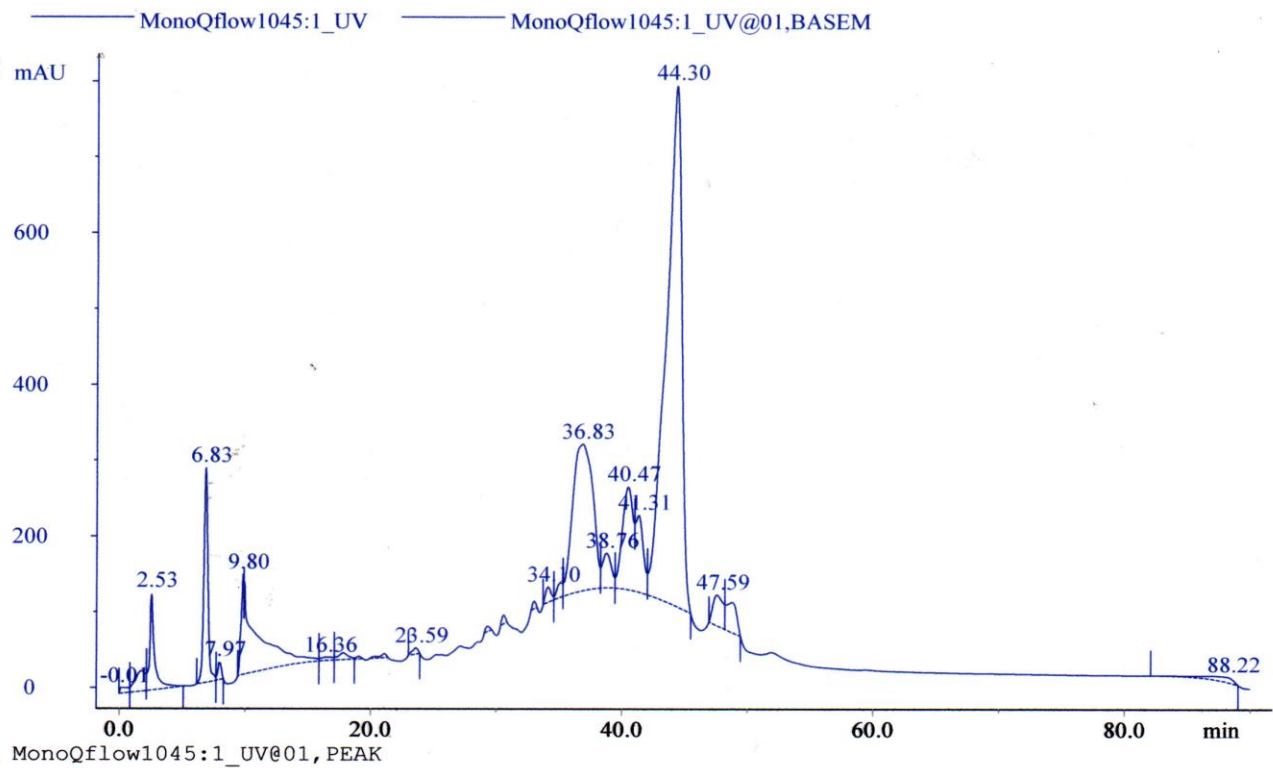


(b) Synthesis scale: 0.2 μ mole, C=0.05 M

DMT data:

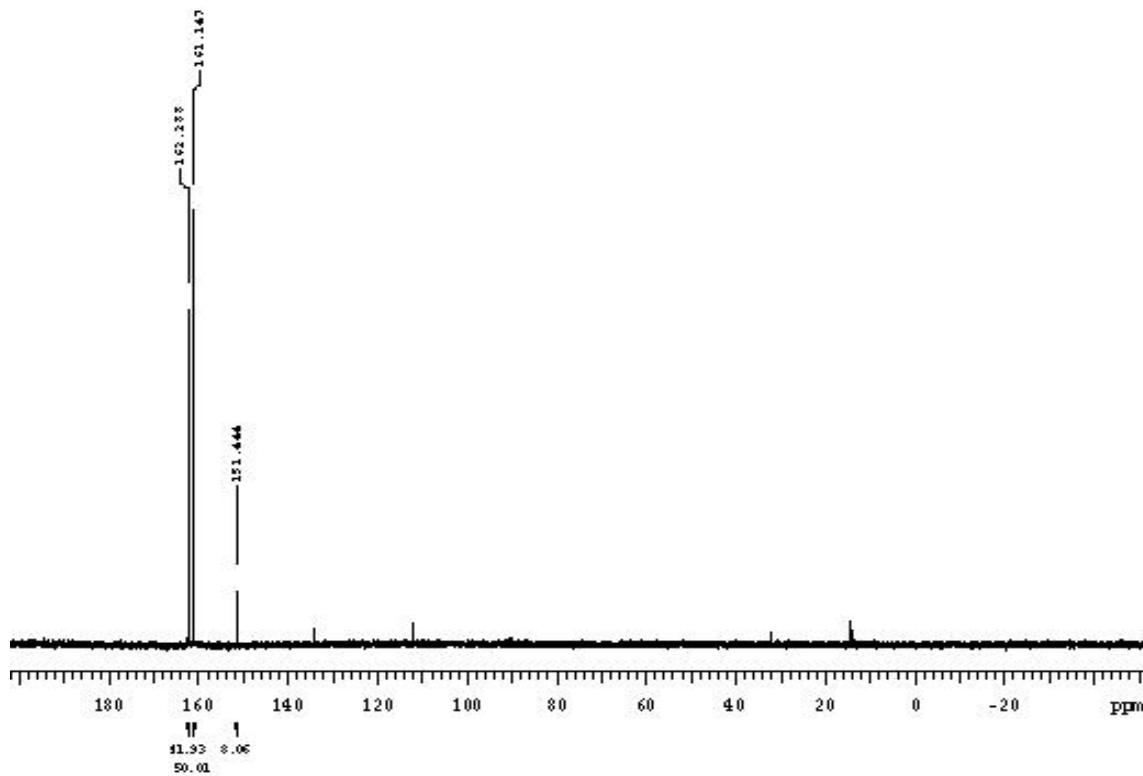


FPLC chromatographic of the crude synthesis



(3) dG-Thiophosphoramidite (Lot#: AM_31):

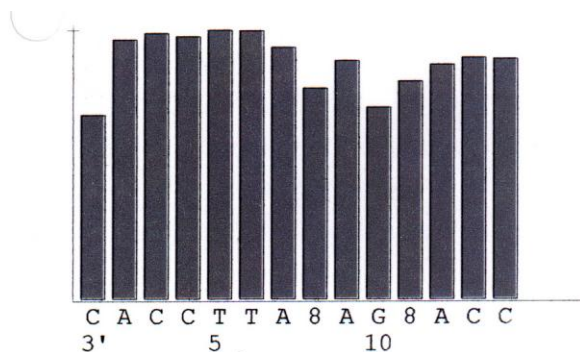
^{31}P -NMR (161MHz) in CD_2Cl_2



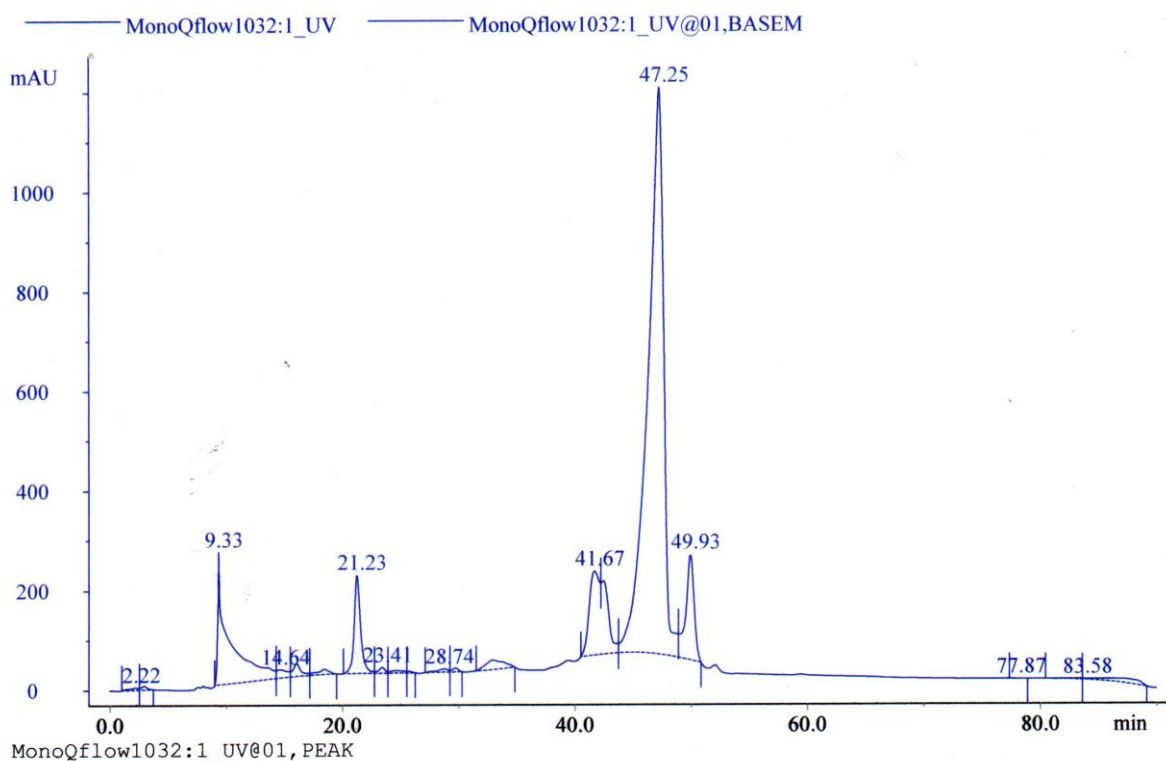
Testing sequence (AA_7): 5'-CCAG_{s2}GAG_{s2}ATTCCAC-3'

(a) Synthesis scale: 1 μ mole, C=0.05 M

DMT data:



FPLC chromatographic of the crude synthesis

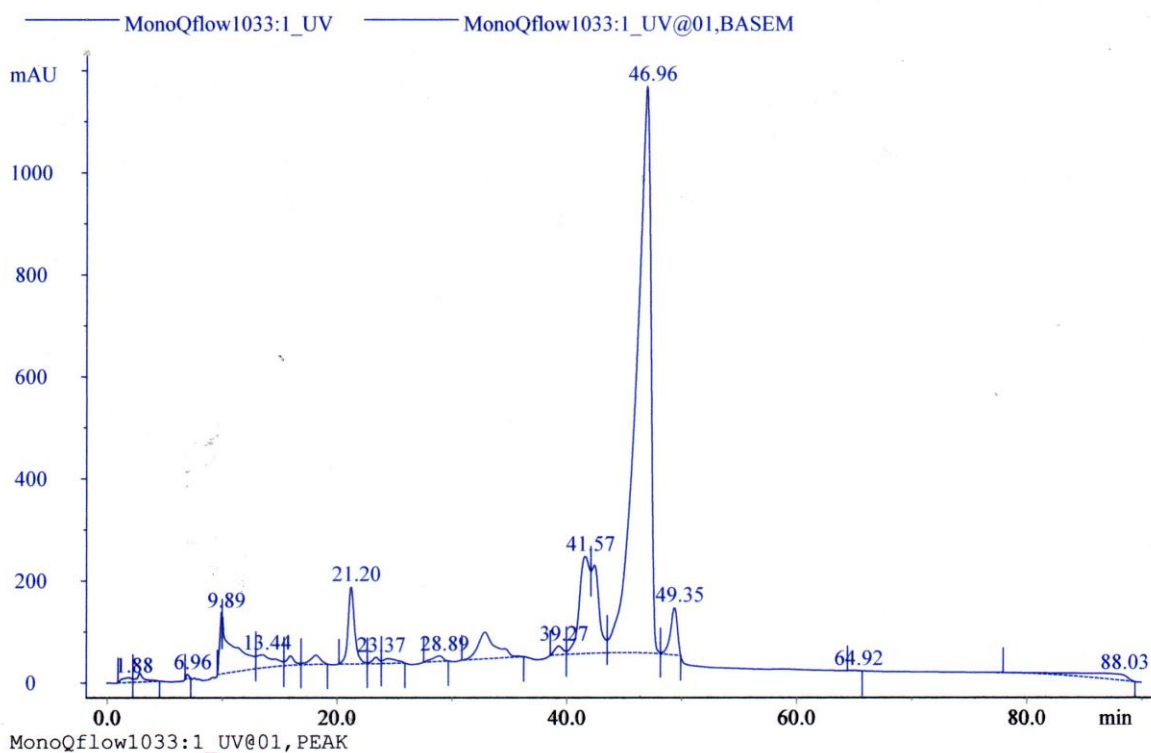


(b) Synthesis scale: 0.2 μ mole, C=0.05 M

DMT data:

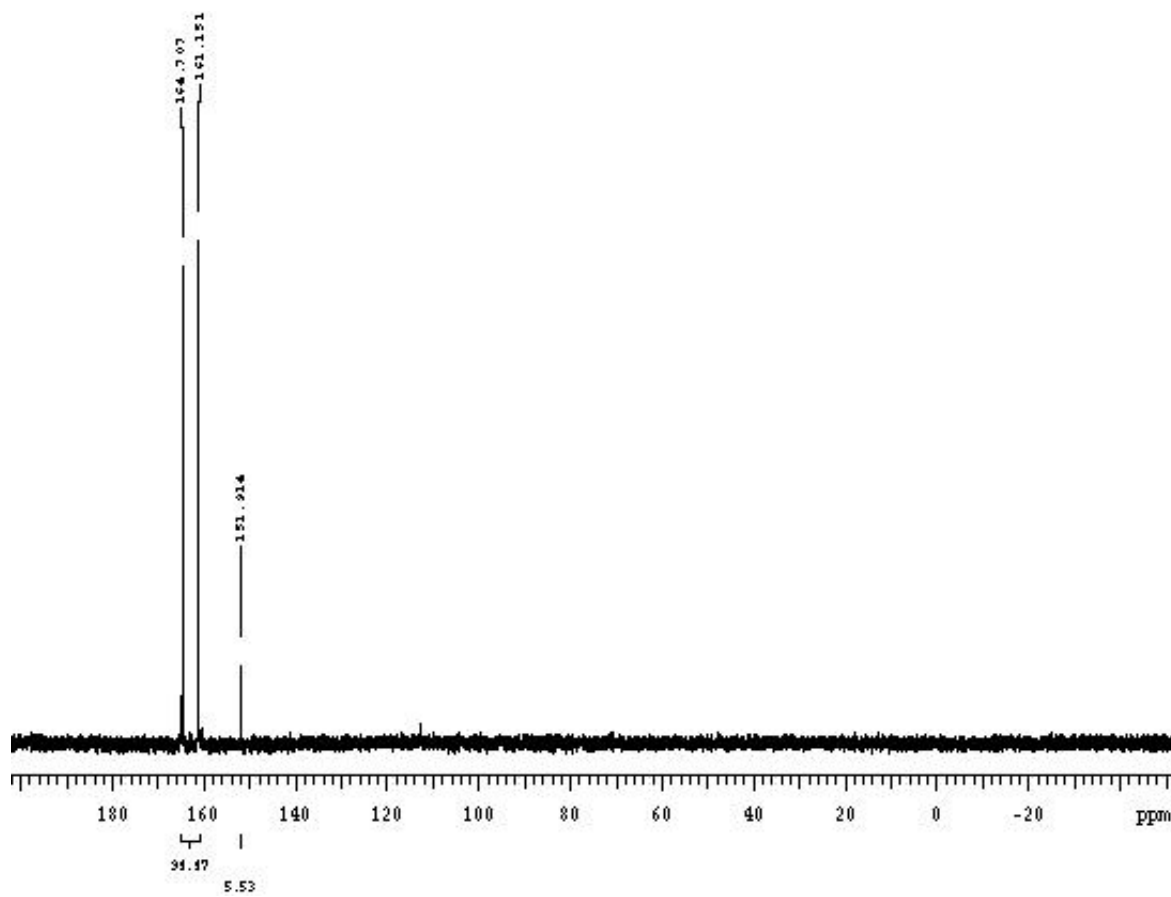


FPLC chromatographic of the crude synthesis



(4) dT-Thiophosphoramidite (Lot#: AM_30):

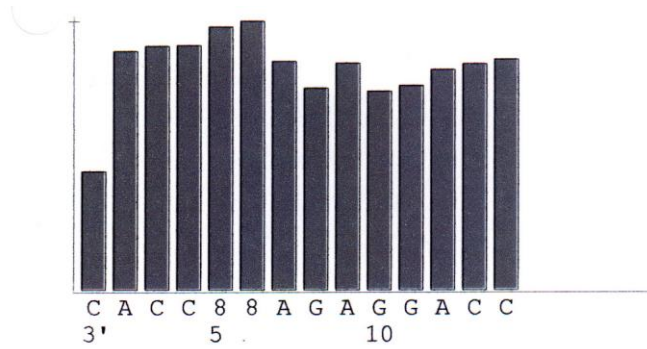
^{31}P -NMR (161MHz) in CD_2Cl_2



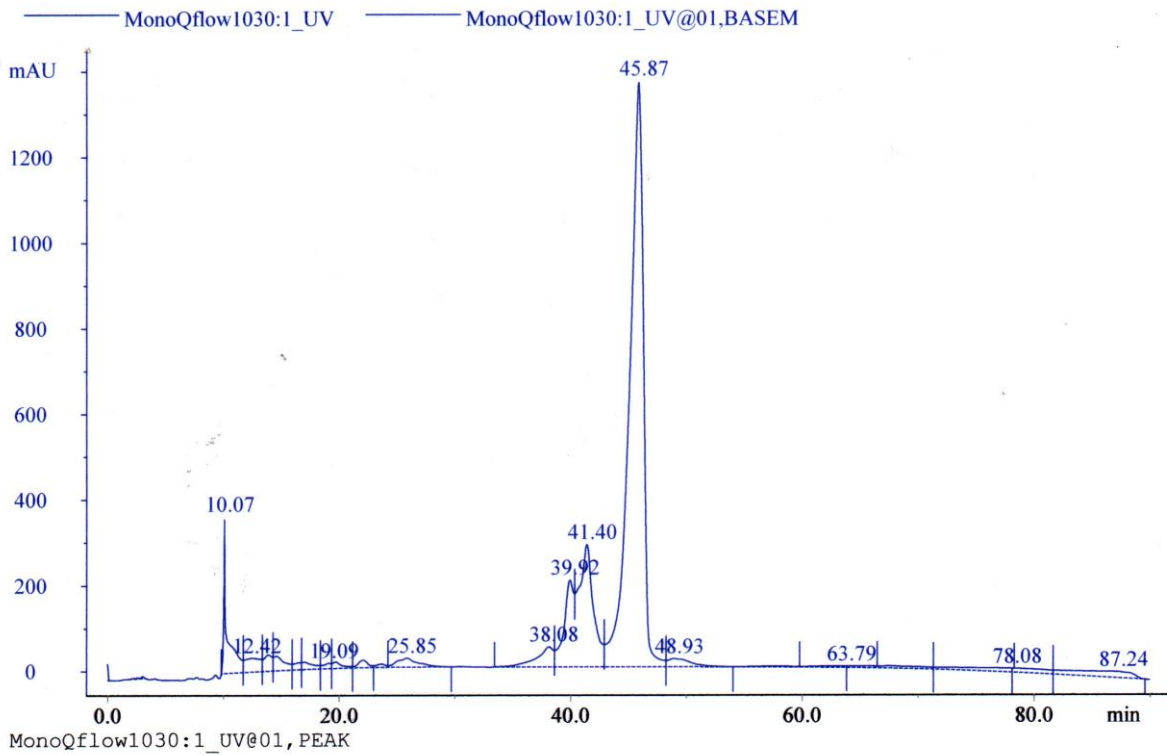
Testing sequence (AA_5): 5'-CCAG GAG AT_{s2}T_{s2}CCAC-3'

(a) Synthesis scale: 1 μ mole, C=0.05 M

DMT data:

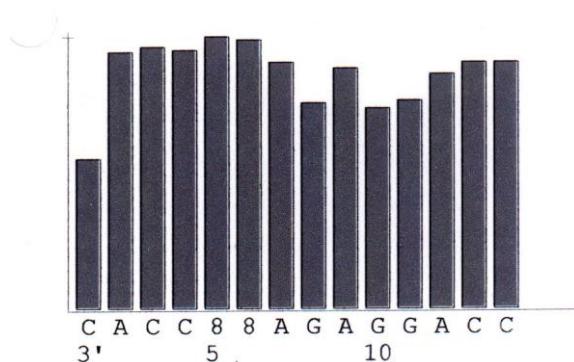


FPLC chromatographic of the crude synthesis

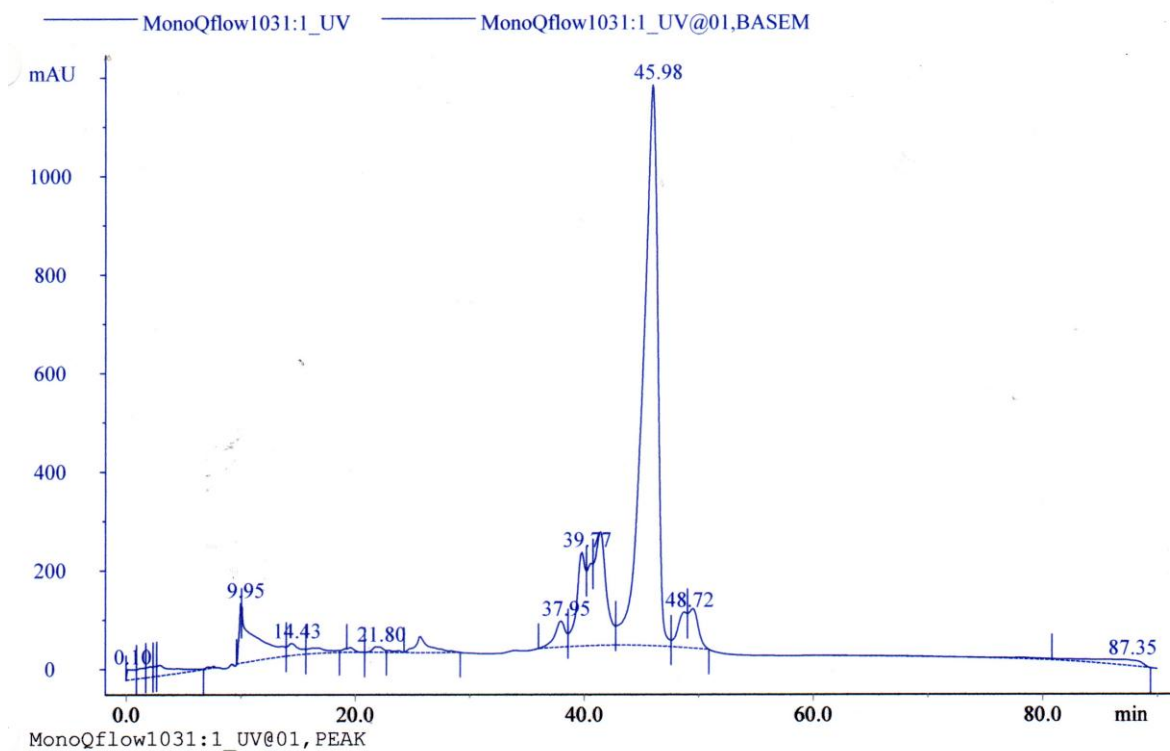


(b) Synthesis scale: 0.2 μ mole, C=0.1 M

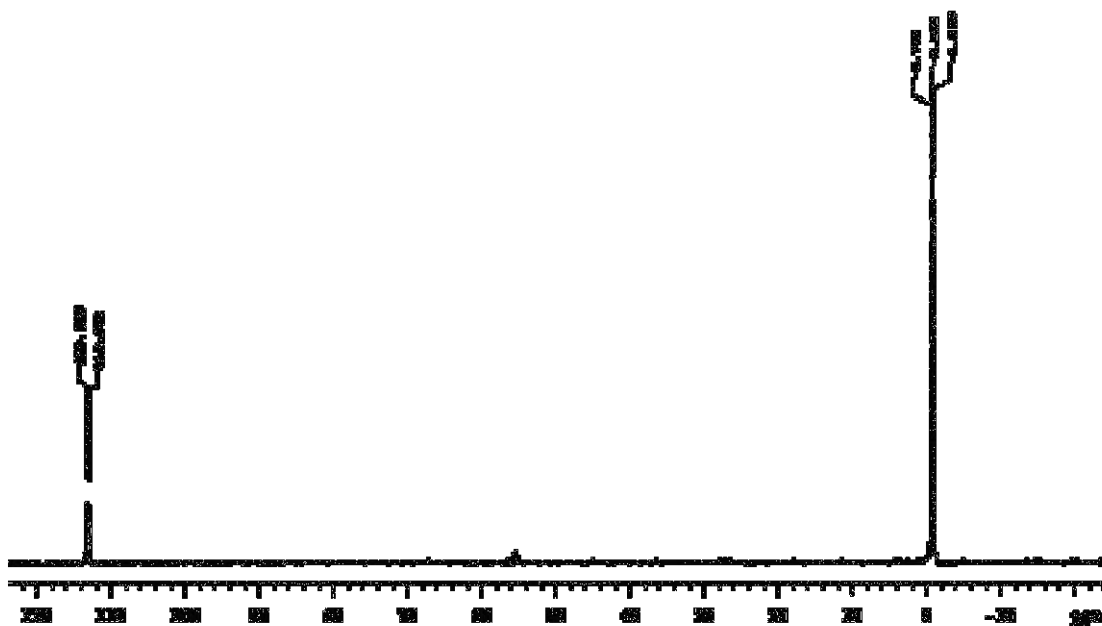
DMT data:



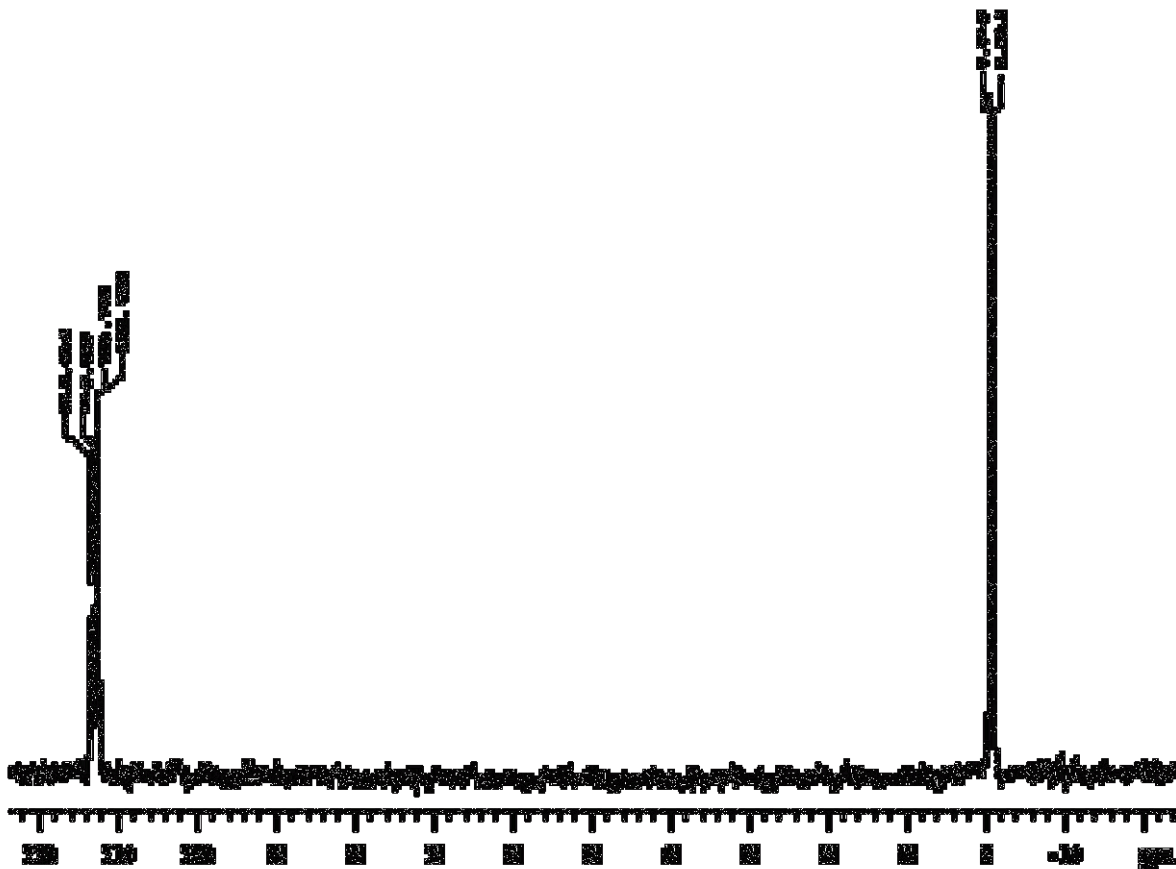
FPLC chromatographic of the crude synthesis



(5) ^{31}P NMR of crude 5'-CCAGGAGATS2TS2CCAC-3'



(6) ^{31}P NMR of purified 5'-GT₂GGA₂AT₂CT₂CCT₂GG-3'



(7) 1 micromole protocol for the synthesis of normal DNA bases (A, C, G and T) on Expedite 8909 Synthesizer.

```
*****
* Protocol Cycle Report: Cycle A (dAdenosine) of "S2_CPG_2_Coup_011107"          Page 1
* Expedite(TM) Nucleic Acid Synthesis System (Workstation)
* Thu Feb 01 11:56:11 2007
*****
```

```
Created:      Thu Jan 11 11:43:01 2007
Modified:    Thu Jan 11 12:11:01 2007
Project:     Expedite System
Author:      PerSeptive Biosystems
Source:      DNA 1 umole Protocol Master
Type:        DNA, normal
Scale:       1 micromole
Comments:    Standard protocol for the synthesis of
              dithiophosphate
```

DNA at the 1 umole scale. The protocol modification was based on DithioCPGTwoCoup050702.

```
/* ----- */
/*      Function          Mode  Amount  Time(sec)      Description      */
/*                               /Arg1  /Arg2                               */
/* ----- */
$Deblocking
144 /*Index Fract. Coll.  */ NA      1      0      "Event out ON"
   0 /*Default            */ WAIT    0      1.5    "Wait"
141 /*Trityl Mon. On/Off */ NA      1      1      "START data collection"
   16 /*Dblk              */ PULSE  10     0      "Dblk to column"
   16 /*Dblk              */ PULSE  50     49     "Deblock"
   38 /*Diverted Wsh A    */ PULSE  40     0      "Flush system with Wsh A"
141 /*Trityl Mon. On/Off */ NA      0      1      "STOP data collection"
   38 /*Diverted Wsh A    */ PULSE  40     0      "Flush system with Wsh A"
144 /*Index Fract. Coll. */ NA      2      0      "Event out OFF"
$Coupling
   1 /*Wsh                */ PULSE  5      0      "Flush system with Wsh"
   2 /*Act                */ PULSE  5      0      "Flush system with Act"
  18 /*A + Act           */ PULSE  5      0      "Monomer + Act to column"
  18 /*A + Act           */ PULSE  2     16     "Couple monomer"
   2 /*Act                */ PULSE  3     24     "Couple monomer"
   1 /*Wsh                */ PULSE  7     56     "Couple monomer"
   1 /*Wsh                */ PULSE  8      0      "Flush system with Wsh"
$Capping
  12 /*Wsh A             */ PULSE  20     0      "Flush system with Wsh A"
  13 /*Caps              */ PULSE  8      0      "Caps to column"
  12 /*Wsh A             */ PULSE  6     15     "Cap"
  12 /*Wsh A             */ PULSE  14     0      "Flush system with Wsh A"
$Oxidizing
  15 /*Ox                */ PULSE  15     5      "Ox to column"
  12 /*Wsh A             */ PULSE  15     0      "Flush system with Wsh A"
$Capping
  13 /*Caps              */ PULSE  7      0      "Caps to column"
  12 /*Wsh A             */ PULSE  30     0      "End of cycle wash"
```

(8) 1 micromole protocol for the synthesis of DITHIO_DNA bases (A, C, G and T) on Expedite 8909 Synthesizer.

```
*****
* Protocol Cycle Report: Cycle 6 (6) of "S2_CPG_2_Coup_011107"                               Page 1 *
* Expedite(TM) Nucleic Acid Synthesis System (Workstation)                               *
* Thu Feb 01 11:56:53 2007                                                             *
*****
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Created:      Thu Jan 11 11:43:01 2007
Modified:    Thu Jan 11 12:11:01 2007
Project:     Expedite System
Author:      PerSeptive Biosystems
Source:      DNA 1 umole Protocol Master
Type:        DNA, normal
Scale:       1 micromole
Comments:    Standard protocol for the synthesis of
              dithiophosphate
```

```
DNA at the 1 umole scale. The protocol
modification
was based on DithioCPGTwoCoup050702.
```

```
/* ----- */
/*      Function                Mode  Amount  Time(sec)  Description      */
/*      /Arg1 /Arg2                                     */
/* ----- */
```

Function	Mode	Amount	Time(sec)	Description
\$Deblocking				
144 /*Index Fract. Coll.	*/ NA	1	0	"Event out ON"
0 /*Default	*/ WAIT	0	1.5	"Wait"
141 /*Trityl Mon. On/Off	*/ NA	1	1	"START data collection"
16 /*Dblk	*/ PULSE	10	0	"Dblk to column"
16 /*Dblk	*/ PULSE	50	49	"Deblock"
38 /*Diverted Wsh A	*/ PULSE	40	0	"Flush system with Wsh A"
141 /*Trityl Mon. On/Off	*/ NA	0	1	"STOP data collection"
38 /*Diverted Wsh A	*/ PULSE	40	0	"Flush system with Wsh A"
144 /*Index Fract. Coll.	*/ NA	2	0	"Event out OFF"
\$Coupling				
1 /*Wsh	*/ PULSE	5	0	"Flush system with Wsh"
2 /*Act	*/ PULSE	5	0	"Flush system with Act"
23 /*6 + Act	*/ PULSE	6	0	"Monomer + Act to column"
23 /*6 + Act	*/ PULSE	1	10	"Couple monomer"
2 /*Act	*/ PULSE	4	32	"Couple monomer"
1 /*Wsh	*/ PULSE	7	56	"Couple monomer"
1 /*Wsh	*/ PULSE	8	0	"Flush system with Wsh"
1 /*Wsh	*/ PULSE	5	0	"Flush system with Wsh"
2 /*Act	*/ PULSE	5	0	"Flush system with Act"
23 /*6 + Act	*/ PULSE	6	0	"Monomer + Act to column"
23 /*6 + Act	*/ PULSE	1	10	"Couple monomer"
2 /*Act	*/ PULSE	4	32	"Couple monomer"
1 /*Wsh	*/ PULSE	7	56	"Couple monomer"
1 /*Wsh	*/ PULSE	8	0	"Flush system with Wsh"
\$Oxidizing				
17 /*Aux	*/ PULSE	15	5	"Aux to column"
1 /*Wsh	*/ PULSE	6	60	"Flush system with Wsh"
17 /*Aux	*/ PULSE	15	5	"Aux to column"
1 /*Wsh	*/ PULSE	6	60	"Flush system with Wsh"
17 /*Aux	*/ PULSE	15	5	"Aux to column"
1 /*Wsh	*/ PULSE	6	60	"Flush system with Wsh"
17 /*Aux	*/ PULSE	15	0	"Aux to column"
1 /*Wsh	*/ PULSE	6	60	"Flush system with Wsh"
12 /*Wsh A	*/ PULSE	9	0	"Flush system with Wsh A"
\$Capping				
12 /*Wsh A	*/ PULSE	20	0	"Flush system with Wsh A"
13 /*Caps	*/ PULSE	8	0	"Caps to column"
12 /*Wsh A	*/ PULSE	6	15	"Cap"
12 /*Wsh A	*/ PULSE	14	0	"Flush system with Wsh A"
12 /*Wsh A	*/ PULSE	30	0	"End of cycle wash"

(9) 0.2 micromole protocol for the synthesis of normal DNA bases (A, C, G and T) on Expedite 8909 Synthesizer.

```
*****
* Protocol Cycle Report: Cycle A (dAdenosine) of "S2_0.2umol_CPG_012407"           Page 1 *
* Expedite(TM) Nucleic Acid Synthesis System (Workstation)                         *
* Thu Feb 01 11:53:38 2007                                                         *
*****
```

```
Created:      Thu Jan 25 11:38:06 2007
Modified:     Thu Jan 25 11:53:47 2007
Project:      Expedite System
Author:       PerSeptive Biosystems
Source:       DNA 0.2 umole Protocol Master
Type:         DNA, normal
Scale:        200 nanomole
Comments:     Protocol for the synthesis of
              DNA Containing dithio-linkages
              at the 0.2 umole scale.
```

```
/* ----- */
/*      Function      Mode  Amount  Time(sec)  Description      */
/*      /Arg1 /Arg2                                     */
/* ----- */
$Deblocking
144 /*Index Fract. Coll. */ NA      1      0      "Event out ON"
0 /*Default             */ WAIT    0      1.5    "Wait"
141 /*Trityl Mon. On/Off */ NA      1      1      "START data collection"
16 /*Dblk               */ PULSE   10     0      "Dblk to column"
16 /*Dblk               */ PULSE   50     49     "Deblock"
38 /*Diverted Wsh A     */ PULSE   40     0      "Flush system with Wsh A"
141 /*Trityl Mon. On/Off */ NA      0      1      "STOP data collection"
144 /*Index Fract. Coll. */ NA      2      0      "Event out OFF"
$Coupling
1 /*Wsh                 */ PULSE   5      0      "Flush system with Wsh"
2 /*Act                 */ PULSE   5      0      "Flush system with Act"
18 /*A + Act           */ PULSE   5      0      "Monomer + Act to column"
2 /*Act                 */ PULSE   5      78     "Couple monomer"
1 /*Wsh                 */ PULSE   1      16     "Couple monomer"
1 /*Wsh                 */ PULSE   14     0      "Flush system with Wsh"
$Capping
12 /*Wsh A              */ PULSE   20     0      "Flush system with Wsh A"
13 /*Caps               */ PULSE   8      0      "Caps to column"
12 /*Wsh A              */ PULSE   6      15     "Cap"
12 /*Wsh A              */ PULSE   14     0      "Flush system with Wsh A"
$Oxidizing
15 /*Ox                 */ PULSE   15     5      "Ox to column"
12 /*Wsh A              */ PULSE   15     0      "Flush system with Wsh A"
$Capping
13 /*Caps               */ PULSE   7      0      "Caps to column"
12 /*Wsh A              */ PULSE   30     0      "End of cycle wash"
```

(10) 0.2 micromole protocol for the synthesis of DITHIO_DNA bases (A, C, G and T) on Expedite 8909 Synthesizer.

```
*****
* Protocol Cycle Report: Cycle 6 (6) of "S2_0.2umol_CPG_012407" Page 1 *
* Expedite(TM) Nucleic Acid Synthesis System (Workstation) *
* Thu Feb 01 11:54:12 2007 *
*****
```

```
Created: Thu Jan 25 11:38:06 2007
Modified: Thu Jan 25 11:53:47 2007
Project: Expedite System
Author: PerSeptive Biosystems
Source: DNA 0.2 umole Protocol Master
Type: DNA, normal
Scale: 200 nanomole
Comments: Protocol for the synthesis of
          DNA Containing dithio-linkages
          at the 0.2 umole scale.
```

```
/* ----- */
/* Function Mode Amount Time(sec) Description */
/* /Arg1 /Arg2 */
/* ----- */
$Deblocking
144 /*Index Fract. Coll. */ NA 1 0 "Event out ON"
0 /*Default */ WAIT 0 1.5 "Wait"
141 /*Trityl Mon. On/Off */ NA 1 1 "START data collection"
16 /*Dblk */ PULSE 10 0 "Dblk to column"
16 /*Dblk */ PULSE 50 49 "Deblock"
38 /*Diverted Wsh A */ PULSE 40 0 "Flush system with Wsh A"
141 /*Trityl Mon. On/Off */ NA 0 1 "STOP data collection"
144 /*Index Fract. Coll. */ NA 2 0 "Event out OFF"
$Coupling
1 /*Wsh */ PULSE 5 0 "Flush system with Wsh"
2 /*Act */ PULSE 5 0 "Flush system with Act"
23 /*6 + Act */ PULSE 5 0 "Monomer + Act to column"
2 /*Act */ PULSE 1 0 "Chase with Act"
2 /*Act */ PULSE 4 63 "Couple monomer"
1 /*Wsh */ PULSE 2 31 "Couple monomer"
1 /*Wsh */ PULSE 13 0 "Flush system with Wsh"
2 /*Act */ PULSE 5 0 "Flush system with Act"
23 /*6 + Act */ PULSE 5 0 "Monomer + Act to column"
2 /*Act */ PULSE 1 0 "Chase with Act"
2 /*Act */ PULSE 4 63 "Couple monomer"
1 /*Wsh */ PULSE 2 31 "Couple monomer"
1 /*Wsh */ PULSE 13 0 "Flush system with Wsh"
$Oxidizing
17 /*Aux */ PULSE 15 5 "Aux to column"
1 /*Wsh */ PULSE 6 60 "Flush system with Wsh"
17 /*Aux */ PULSE 15 5 "Aux to column"
1 /*Wsh */ PULSE 6 60 "Flush system with Wsh"
17 /*Aux */ PULSE 15 5 "Aux to column"
1 /*Wsh */ PULSE 6 60 "Flush system with Wsh"
17 /*Aux */ PULSE 15 0 "Aux to column"
1 /*Wsh */ PULSE 6 60 "Flush system with Wsh"
12 /*Wsh A */ PULSE 9 0 "Flush system with Wsh A"
$Capping
12 /*Wsh A */ PULSE 20 0 "Flush system with Wsh A"
13 /*Caps */ PULSE 8 0 "Caps to column"
12 /*Wsh A */ PULSE 6 15 "Cap"
12 /*Wsh A */ PULSE 14 0 "Flush system with Wsh A"
12 /*Wsh A */ PULSE 30 0 "End of cycle wash"
```