

RNA synthesis using 2'-thiomorpholine-4-carbothioate (TC) protection

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Overview

TC-RNA chemistry has a one-step deprotection method that removes both the nucleobase and 2'-OH protection simultaneously in 2-4 hours at room temperature with anhydrous ethylenediamine/toluene 1:1. As with AMA, the use of ethylenediamine will lead to transamination if used with Bz-C or Bz-dC therefore TC-C is acetyl protected (**2180**) and Ac-dC (**2034**) is used when synthesising DNA/RNA chimera.

Four 2'-TC-protected monomers are available (**2177-2180**). Note that standard RNA supports, as used with TBDMS chemistry, are compatible with TC chemistry (the 2'-OH is Ac-protected).

Use Protocol

Note that detailed instrument cycle files are available on our web site to download.

Dissolution

All monomers are diluted with anhydrous toluene (**4055**). Anhydrous, alcohol-free DCM/MeCN (1:4) can also be used, however it should be noted that the amidites can precipitate from solution in cooler conditions (<18°C). See the table below for dilution data. Both 0.1M (ABI/MerMade) and 0.067M (Expedite) data is given, though we recommend using 0.1M solutions.

We recommend the use of 4Å or 3Å molecular sieves which have been prepared in the following way:

1. Pour the sieves into a Büchner funnel with no filter paper (but make sure the holes are small enough that the sieves don't fall into them).
2. Wash them with:

- Acetonitrile
 - DCM
 - 1% oxalic acid in DCM (don't use deblock)
 - DCM
 - Acetonitrile
 - 2% DIPEA (or TEA) in MeCN
 - Acetonitrile
3. Dry in an oven at 150-200°C for 72h.
 4. Store in a vacuum dessicator.

Coupling

3-5min is recommended. Use 0.5M ETT as activator (**0237/3145/3146**).

Cleavage & Deprotection

Separate procedures for use of both a vacuum manifold and filter tubes are provided (however, we recommend the use of a manifold):

(a) Vacuum Manifold

Diethylamine wash

1. At the end of the synthesis dry the resin by flushing argon through the column.
2. Wash the resin for 3min with 20% diethylamine in dry acetonitrile (either on the synthesiser or on a vacuum manifold).
3. Dry the resin by flushing argon through the column (or by drying under vacuum if using a manifold).
4. Wash with dry MeCN three times with drying between each wash.

Ethylene diamine deprotection

5. Place the columns on a vacuum manifold with a 3ml syringe barrel on the top of each column.

Continued ...

Item No.	Mol. Wt.	Unit Wt.	Dilution (0.1M)/ml			Dilution (0.067M)/ml		
			250mg	500mg	1g	250mg	500mg	1g
2177	924.03	306.17	2.71	5.41	10.82	4.04	8.08	16.15
2178	1033.16	345.21	2.42	4.84	9.68	3.61	7.22	14.45
2179	1051.18	329.21	2.38	4.76	9.51	3.55	7.10	14.20
2180	965.09	305.18	2.59	5.18	10.36	3.87	7.73	15.47

6. Turn the vacuum on to ensure the resin is dry.
7. Turn the vacuum off and close all the taps to the column positions.
8. Add 50% ethylene diamine in toluene (500µl) to the syringe barrel.
9. Turn the vacuum on.
10. One column at a time, open the tap to allow the solution to come through the column but close the tap before the solution comes through the tap.
11. Turn off the vacuum.
12. Leave at room temperature for 2-4h depending on the length and GC content of the oligo.
13. Turn the vacuum on and open the taps to the columns to remove the EDA/toluene solution.
14. Turn the vacuum off.
15. Add 2.5ml of dry acetonitrile to the syringe barrel.
16. Turn the vacuum on to remove the acetonitrile.
17. Repeat the acetonitrile wash.
18. Dry the resin under vacuum for 2min.
19. Turn off the vacuum.
20. Place an appropriately labelled tube (RNase free) under each of the columns.
21. Add at least 0.5ml (but typically 1ml) of 0.1M TEAA, pH 7.0 prepared with DEPC treated RNase free water (or other suitable buffer being used in the purification step).
22. Turn on the vacuum to elute the product into the tube.
23. Desalt the oligo by G25. The sample is now ready for purification.

(b) Filter Tubes (0.22µm)

Diethylamine wash

1. At the end of the synthesis dry the resin by flushing argon through the column.
2. Wash the resin for 3min with 20% diethylamine in dry acetonitrile (either on the synthesiser or on a vacuum manifold).
3. Dry the resin by flushing argon through the column (or by drying under vacuum if using a manifold).
4. Wash with dry MeCN three times with drying between each wash.

Ethylene diamine deprotection

5. Empty the resin into the filter part of the filter tube. (Note it is important that the filter tube being used does not allow the deprotection solution to flow through the filter until placed in the centrifuge), an alternative is to place the resin in a 2ml sample tube and add 500µl of the deprotection solution.
6. Add 500µl EDA/toluene (1:1).
7. Leave at room temperature for 2-4h depending on the length and GC content of the oligo.
8. If using a sample tube rather than a filter tube, transfer the contents of the sample tube to the filter part of the filter tube.
9. Centrifuge for 10min at 9000rpm to remove the EDA/toluene solution.
10. Discard the eluant
11. Wash with MeCN
12. Centrifuge for 10min at 9000rpm.
13. Discard the eluant
14. Wash with MeCN three more times.

15. Add at least 0.5ml (but typically 1ml) 0.1M TEAA, pH7.0 prepared with DEPC treated RNase free water (500µl) (or any other suitable buffer being used in the purification step) and centrifuge for 10min at 9000rpm to elute the oligo from the resin.

16. Desalt the oligo by G25. The sample is now ready for purification.

Purification

Use standard RNA purification methods.

Storage & Stability

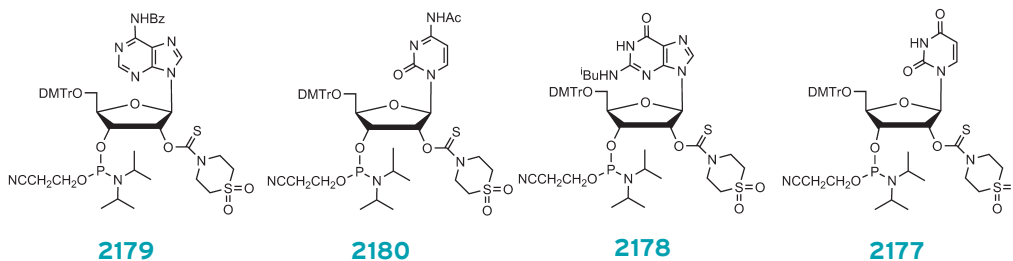
Solutions must be prepared freshly and used within 72h. Monomers must be stored at -10 to -30°C.

Unlike DNA, RNA is highly unstable in basic media and must not be exposed to high pH. Traces of heavy metal ions present in various salts used also lead to degradation. Buffers containing 1mM EDTA are used to prevent this.

RNA is very sensitive to degradation by nucleases. All glass (and plastic) used must be rinsed in water containing diethylpyrocarbonate (DEPC) (2%) and autoclaved before re-use. All reagents, water and consumables used in subsequent handling must be sterile and certified RNase free. Anion-exchange and desalting columns should be stored in sterile water/MeCN (1:1), except Q-res type which should be stored in water/ethanol (4:1).

Further Information

For up to date ordering and protocol information please see www.linktech.co.uk, e-mail us at sales@linktech.co.uk or call +44(0)1698 849911.



Ordering 2'-TC RNA Phosphoramidites & Toluene Diluent

Product	Pack Size	Cat. No.	Product	Pack Size	Cat. No.
Bz-A-TC-RNA CE	250mg	2179-B250	iBu-G-TC-RNA CE	250mg	2178-B250
Phosphoramidite	500mg	2179-B500	Phosphoramidite	500mg	2178-B500
	1g	2179-C001		1g	2178-C001
Ac-C-TC-RNA CE	250mg	2180-B250	U-TC-RNA CE	250mg	2177-B250
Phosphoramidite	500mg	2180-B500	Phosphoramidite	500mg	2177-B500
	1g	2180-C001		1g	2177-C001
			Diluent (Toluene, anhydrous)	100ml	4055-D100

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