Cleavage Cocktail Selection

After a peptide has been synthesized on a solid support resin, and any required on-resin modifications have been made, the peptide can be cleaved from the resin. Trifluoracetic acid (TFA) is the main reagent utilized to perform this final cleavage of the peptide from resin together with the removal of the side chain protecting groups. In this process, highly reactive carbocations are generated and it is necessary to trap them to avoid undesired reactions with sensitive amino acids. Therefore, different scavengers are added to the TFA based on the amino acid residues and associated protecting groups present on the peptide. Cleavage of tert-butyl and Boc sidechain protecting groups leads to tert-butyl cations that form by the S_N1-deprotection mechanism and tert-butyl trifluoroacetate.[1] There are a variety of different cleavage cocktails that have been recommended for use. **Table 1** lists a few common cleavage cocktails: Reagents B, H, I, K, L, and R. Any one of these cleavage cocktails can be used if the following residues are NOT present:

- Arg(Mtr)
- Arg(Pmc)
- Asn(Mbh)
- Asn(Tmob)
- Gln(Mbh)
- Gln(Tmob)
- Any Trp Residue
- Any Met Residue
- Any Cys Residue
- Any His Residue

If one of the listed amino acid residues are present, then Reagent B or Reagent R should be used.

Table 1 – A selection of different cleavage cocktails for cleaving peptide off solid-support resin.

Cleavage Cocktail	Recipe	Comments
B	TFA/Phenol/Water/TIPS (88/5/5/2)	All peptides.[2]
H	TFA/Phenol/Thioanisole/EDT/Water/Dimethyl Sulfide/Ammonium Iodide (81/5/5/2.5/3/2/1.5)	Helps prevent methionine oxidation.[3]
I	TFA/TIPS/DMB (92.5/2.5/5)	Prevents Rink amide linker decomposition.[4]
K	TFA/Phenol/Water/Thioanisole/EDT (82.5/5/5/5/2.5)	Helpful for sensitive residues.[5]
L	TFA/TIPS/DTT/Water (88/5/5/2)	All peptides.[6]
R	TFA/Thioanisole/EDT/Anisole (90/5/3/2)	All peptides, multiple Arg residues.[7]

* Abbreviations: TFA, trifluoroacetic acid; TIPS, triisopropylsilane; EDT, 1,2-Ethanedithiol; DMB, 1,3-Dimethoxybenzene; DTT, Dithiothreitol

When adding the cleavage cocktail, it is recommended that the reaction be stirred at room temperature for at least two hours and no more than four hours. It is strongly advised that sample cleavages are performed with different cleavage cocktails and to compare the qualitative and quantitative recovery before choosing the final one. The monitoring of these cleavages is best done with analytical HPLC. It is also recommended that a simultaneous time course evaluation of the cleavage be performed to determine the best amount of time to allow the reaction to proceed. ***If short on time, it is recommended to use Reagent B for three hours.***

Tables 2-6 provide the component mass and/or volumes required to make one milliliter of each cleavage cocktail listed in **Table 1**. The highlighted cell represents the mass or volume required for the given component. It is recommended that for one gram of resin being cleaved, 10-20 mL of cleavage cocktail solution be added.[8] Depending on the loading of the resin and the scale of the synthesis, the volume of cleavage cocktail varies significantly. ***As a general rule of thumb, for a 0.10 mmol scale synthesis, 5 mL of cleavage cocktail solution should be added. For a 0.25 mmol scale synthesis, 10 mL of cleavage cocktail solution should be added.***

Table 2 – The cleavage cocktail components and amounts for making one milliliter of Reagent B.[2]

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	930	0.88
Phenol	60	1.070	56.07	0.05
Triisopropylsilane (TIPS)		0.773	20	0.02
Water		1.000	50	0.05

Table 3 – The cleavage cocktail components and amounts for making one milliliter of Reagent H.[3]

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	920	0.81
Phenol	60	1.070	56.07	0.05
Thioanisole	60	1.053	56.96	0.05
1,2-Ethanedithiol (EDT)		1.120	30	0.03
Water		1.000	30	0.03
Dimethyl Sulfide		0.845	20	0.02
Ammonium Iodide	45	2.510	17.93	0.02

Table 4 – The cleavage cocktail components and amounts for making one milliliter of Reagent I.[4]

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	970	0.93
Triisopropylsilane (TIPS)		0.773	30	0.03
1,3-Dimethoxybenzene (DMB)	50	1.053	47.48	0.05

Table 5 – The cleavage cocktail components and amounts for making one milliliter of Reagent K.[5]

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	915	0.82
Phenol	60	1.070	56.07	0.05
Water		1.000	55	0.05
Thioanisole	60	1.053	56.96	0.05
1,2-Ethandedithiol (EDT)		1.120	30	0.03

Table 6 – The cleavage cocktail components and amounts for making one milliliter of Reagent L.[6]

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	910	0.88
Triisopropylsilane (TIPS)		0.773	55	0.05
Dithiothreitol (DTT)	65	1.377	47.20	0.05
Water		1.000	25	0.02

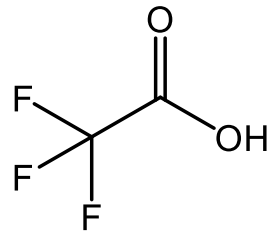
Table 7 – The cleavage cocktail components and amounts for making one milliliter of Reagent R.[7]

Component	Mass (mg)	Density (g/mL)	Volume (µL)	Percentage
Trifluoroacetic acid (TFA)		1.490	950	0.90
Thioanisole	60	1.053	56.98	0.05
1,2-Ethanedithiol (EDT)		1.120	320	0.03
Anisole		0.995	20	0.02

Scavengers

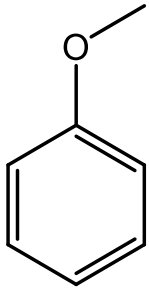
The different scavengers have different reactive groups that they sequester in the cleavage milieu. Some of the more common scavengers are listed below with information about their scavenging properties.

Trifluoroacetic acid (TFA):



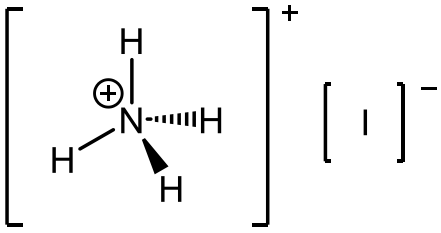
Trifluoroacetic acid is responsible for the deprotection of sidechain protecting groups and cleavage of the synthesized peptide from the solid support resin. Both occur in one single step.

Anisole:



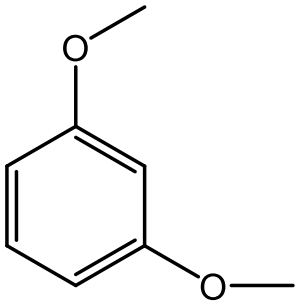
Anisole is usually used in conjunction with EDT to help in the prevention of alkylation of Cysteine by Tryptophan.[1,9] This can also be used to scavenge tert-butyl groups.[10]

Ammonium Iodide:



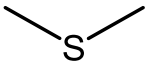
Ammonium iodide is a thiol reducing agent that can react with oxidized Methionine to return the residue to its proper form.[3] It is used in conjunction with TFA and dimethyl sulfide to reduce the Methionine sulfoxide to Methionine.

1,3-Dimethoxybenzene (DMB):



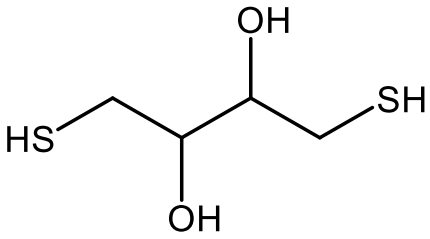
1,3-Dimethoxybenzene is used to prevent the formation of a C-terminal N-alkylated amide by-product when cleaving Rink amide resin.[4] Only certain peptide sequences are prone to this by-product formation.

Dimethyl Sulfide:



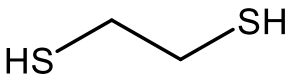
Dimethyl sulfide, in conjunction with TFA and ammonium iodide, is required for the reduction of Methionine sulfoxide to Methionine.[3]

Dithiothreitol (DTT):



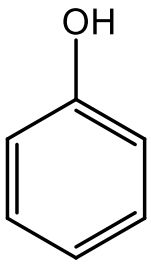
Dithiothreitol is a reducing agent scavenger that reduces disulfide bonds in peptides and proteins. This is important in preventing inter- and intra-molecular disulfide bonds forming between Cysteine residues. When performing a reduction reaction, it is often important for the reaction to proceed in denaturing conditions because DTT cannot reduce buried (or solvent-inaccessible) disulfide bonds.

1,2-Ethanedithiol (EDT):



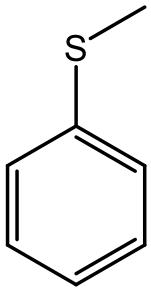
1,2-Ethanedithiol is the most common and efficient scavenger. It has been shown to be the most efficient tert-butyl trifluoroacetate and HMP linker scavenger,[1,10] as well as being helpful in the prevention of alkylation of Cysteine by Tryptophan (with the supplement of anisole).[5,9]

Phenol:



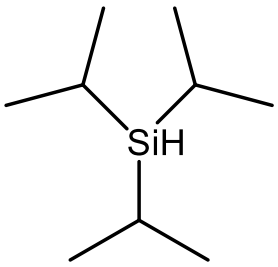
Phenol is a scavenger that has been shown to preserve the integrity of peptides containing Tryptophan and/or Tyrosine residues.[5] It is also helpful in the prevention of Pmc or Pbf group modification to the peptide.[2] Phenol also scavenges tert-butyl groups.[10]

Thioanisole:



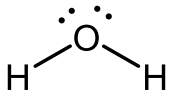
Thioanisole, a reducing agent, has been shown to prevent the oxidation of the thioether in the Methionine residue.[5] It is also an efficient scavenger of the Bzl group.[1]

Triisopropylsilane (TIPS):



Triisopropylsilane is a reducing agent scavenger that is known to scavenge electrophilic species. These electrophilic species are generated from the acid catalyzed deprotection of sidechain groups such as Boc, Pbf, tert-butyl, and trityl. These species must be scavenged or nucleophilic residues, such as those on C, K, R, and S residues, could be attacked. TIPS has been shown to successfully replace the malodorous EDT while showing good efficacy for quenching carbocations in sequences containing R and W.[2,9,11] It is also useful in preventing trityl reattachment to Cysteine.[1,9]

Water (H_2O):



Water is often added to a cleavage cocktail solution to act as a scavenger for tert-butyl cations.[1,5] This becomes most important when D, E, S, T, and Y residues are present in the peptide since they often contain the tert-butyl sidechain protecting group. It can often be used as a single scavenger with peptides that only contain protected C, M, and W residues.

References:

(1) Fields, G. B.; Noble, R. L. Solid Phase Peptide Synthesis Utilizing 9-Fluorenylmethoxycarbonyl Amino Acids. *Int. J. Pept. Protein Res.* **1990**, *35* (3), 161–214. https://doi.org/10.1111/j.1399-3011.1990.tb00939.x.

(2) Sole, N. A.; Barany, G. Optimization of Solid-Phase Synthesis of [Ala8]-Dynorphin A. *J. Org. Chem.* **1992**, *57* (20), 5399–5403. https://doi.org/10.1021/jo00046a022.

(3) Huang, H.; Rabenstein, D. L. A Cleavage Cocktail for Methionine-Containing Peptides. *J. Pept. Res.* **1999**, *53* (5), 548–553. https://doi.org/10.1034/j.1399-3011.1999.00059.x.

(4) Stathopoulos, P.; Papas, S.; Tsikaris, V. C-Terminal N-Alkylated Peptide Amides Resulting from the Linker Decomposition of the Rink Amide Resin. A New Cleavage Mixture Prevents Their Formation. *J. Pept. Sci.* **2006**, *12* (3), 227–232. https://doi.org/10.1002/psc.706.

(5) King, D. S.; Fields, C. G.; Fields, G. B. A Cleavage Method Which Minimizes Side Reactions Following Fmoc Solid Phase Peptide Synthesis. *Int. J. Pept. Protein Res.* **1990**, *36* (3), 255–266. https://doi.org/10.1111/j.1399-3011.1990.tb00976.x.

(6) Bonner, A. G.; Udell, L. M.; Creasey, W. A.; Duly, S. R.; Laursen, R. A. Solid-Phase Precipitation and Extraction, a New Separation Process Applied to the Isolation of Synthetic Peptides. *J. Pept. Res.* **2001**, *57* (1), 48–58. https://doi.org/10.1034/j.1399-3011.2001.00801.x.

(7) Angell, Y. M.; Alsina, J.; Barany, G.; Albericio, F. Practical Protocols for Stepwise Solid-Phase Synthesis of Cysteine-Containing Peptides. *J. Pept. Res.* **2002**, *60* (5), 292–299. https://doi.org/10.1034/j.1399-3011.2002.02838.x.

(8) Amblard, M.; Fehrentz, J.-A.; Martinez, J.; Subra, G. Methods and Protocols of Modern Solid Phase Peptide Synthesis. *Mol. Biotechnol.* **2006**, *33* (3), 239–254. https://doi.org/10.1385/MB:33:3:239.

(9) Fields, C. G.; Fields, G. B. Minimization of Tryptophan Alkylation Following 9-Fluorenylmethoxycarbonyl Solid-Phase Peptide Synthesis. *Tetrahedron Lett.* **1993**, *34* (42), 6661–6664. https://doi.org/10.1016/S0040-4039(00)61669-6.

(10) Lundt, B. F.; Johansen, N. L.; Vølund, A.; Markussen, J. Removal of T-Butyl and t-Butoxycarbonyl Protecting Groups with Trifluoroacetic Acid. *Int. J. Pept. Protein Res.* **1978**, *12* (5), 258–268. https://doi.org/10.1111/j.1399-3011.1978.tb02896.x.

(11) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. Trialkylsilanes as Scavengers for the Trifluoroacetic Acid Deblocking of Protecting Groups in Peptide Synthesis. *Tetrahedron Lett.* **1989**, *30* (21), 2739–2742. https://doi.org/10.1016/S0040-4039(00)99113-5.