

dPEG<sup>®</sup>  
Biotinylation  
Reagents



## Product Features and Benefits:

- x = 4, 12 or 24
- A highly hydrophilic biotinylation reagent with various spacer options
- Eliminate the common issues of aggregation with biotinylated proteins and oligo. Gives ideal spacer properties for optimal streptavidin binding
- Amine reactive biotinylation reagent with a dPEG<sup>®</sup> pegylation spacer arm; activate in situ
- Very water soluble, hydrophilic and eliminates non-specific binding.
- Non-antigenic and non-immunogenic spacer arm
- The dPEG<sup>®</sup> pegylation spacer and its amazing properties ELIMINATES aggregation and precipitation when labeling antibodies and other biological materials, and will also significantly increase S/N in analytical applications as well.
- EXPECT GREAT, even dramatic S/N increases!

## Protocol:

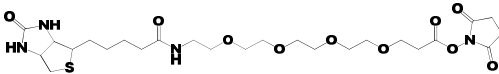
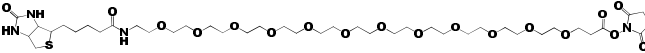
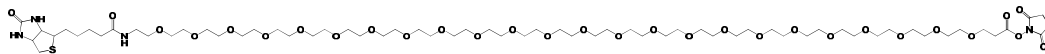
See Hermanson pg. 728 for a typical protocol for a antibody or protein biotinylation using an in situ activated form of 10197. Generally customers use this when they have their favorite way of activating the acid, as opposed to using our dPEG<sup>®</sup><sub>12</sub> pegylation spacer containing biotin reagent. The reagent can be pre-dissolved in an organic solvent OR can be directly dissolved in your reaction medium based on its inherent water solubility. Also, see above in situ activation procedure.

## Reference:

Greg T. Hermanson, Bioconjugate Techniques, 2<sup>nd</sup> Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). Specifically see pp. 726-730 in his Chapter 18 on discrete PEG compounds for pegylation applications.

## Protocol for in situ activation to the NHS ester:

Use a 10-20% molar excess of EDC and NHS in dry methylene chloride (dried over 3A molecular sieves). Add a methylene chloride solution of the acid to the dry reagents under dry conditions. Stir for several hours or overnight, then evaporate the solvent and use. Can also treat reaction mixture with a small amount of silica gel to adsorb the excess EDC and the urea by-product, filter, then evaporate the solvent and use. Note of caution: The NHS should be added with the EDC to prevent formation of the anhydride. Also, can be used for peptide labeling using a variety of activating "concoctions," which can also be used for direct coupling to other amines.

Product #	Description	50 mg	1000 mg
10200	NHS-dPEG <sup>®</sup> <sub>4</sub> -biotin	\$100	\$750
 <p>Mol. Wt.: 588.67; single compound dPEG<sup>®</sup> Spacer is 16 atoms and 19.2 Å</p>			
10198	NHS-dPEG <sup>®</sup> <sub>12</sub> -biotin	\$150	\$1250
 <p>Mol. Wt.: 941.09; single compound dPEG<sup>®</sup> Spacer is 40 atoms and 47.6 Å</p>			
10774	NHS-dPEG <sup>®</sup> <sub>24</sub> -biotin	\$250	\$1400
 <p>Mol. Wt.: 1469.72; single compound dPEG<sup>®</sup> Spacer is 81 atoms and 97.7 Å</p>			



## Product Features and Benefits:

- x = 4, 12, 24, or 48
- Unactivated option
- Imparted physical properties are the biotin same as the dPEG<sup>®</sup><sub>4</sub> NHS esters (10200, 10198); the labeled compound becomes very hydrophilic because of the dPEG<sup>®</sup> pegylation spacer, making the biotin very available for streptavidin binding in the capture/binding step.
- The 10199 pegylation spacer is the same length as 10200 and 10198, resp., and its length is optimal for rapid and tight avidin/SA binding properties.
- Very water soluble, hydrophilic and eliminates non-specific binding.
- 10198 has a LONGER 47.6 Angstrom spacer: the longer pegylation arm gives ready accessibility to the biotin by the streptavidin/avidin conjugate binding; also where the amine of the biotinylation site may be buried, the longer arm is ideal for giving accessibility to the binding conjugates.
- Non-antigenic and non-immunogenic spacer arm
- The dPEG<sup>®</sup> pegylation spacer and its amazing properties ELIMINATES aggregation and precipitation when labeling antibodies and other biological materials, and will also significantly increase S/N in analytical applications as well.

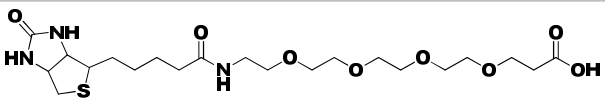
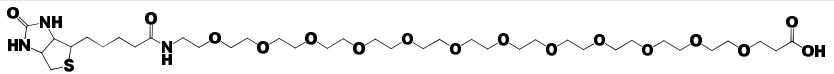
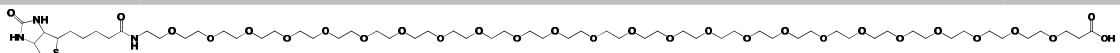
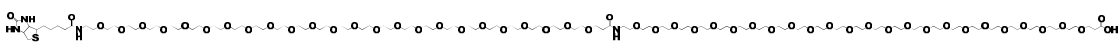
## Protocol for in situ activation to the NHS ester:

Use a 10-20% molar excess of EDC and NHS in dry methylene chloride (dried over 3A molecular sieves). Add a methylene chloride solution of the acid to the dry reagents under dry conditions. Stir for several hours or overnight, then evaporate the solvent and use. Can also treat reaction mixture with a small amount of silica gel to adsorb the excess EDC and the urea by-product, filter, then evaporate the solvent and use.

**Note of caution:** The NHS should be added with the EDC to prevent formation of the anhydride. Also, can be used for peptide labeling using a variety of activating "concoctions," which can also be used for direct coupling to other amines.

## Protocol:

See Hermanson pg. 728 for a typical protocol for a antibody or protein biotinylation using an in situ activated form of 10197. Generally customers use this when they have their favorite way of activating the acid, as opposed to using our dPEG<sup>®</sup><sub>12</sub> pegylation spacer containing biotin reagent. The reagent can be pre-dissolved in an organic solvent OR can be directly dissolved in your reaction medium based on its inherent water solubility. Also, see above in situ activation procedure.

Product #	Description	100 mg	1000 mg
10199	dPEG <sup>®</sup> <sub>4</sub> -biotin acid	\$100	\$600
 <p>Mol. Wt.: 491.60; single compound dPEG<sup>®</sup> Spacer is 16 atoms and 19.2 Å</p>			
10197	dPEG <sup>®</sup> <sub>12</sub> -biotin acid	\$150	\$975
 <p>Mol. Wt.: 844.02; single compound dPEG<sup>®</sup> Spacer is 40 atoms and 47.6 Å</p>			
10773	dPEG <sup>®</sup> <sub>24</sub> -biotin acid	\$225	\$1350
 <p>Mol. Wt.: 1372.65; single compound dPEG<sup>®</sup> Spacer is 76 atoms and 95.7 Å</p>			
10776	dPEG <sup>®</sup> <sub>48</sub> -biotin acid	\$300	\$1500
 <p>Mol. Wt.: 2500.99; single compound dPEG<sup>®</sup> Spacer is 157 atoms and 187.8 Å</p>			



## Product Features and Benefits:

- Alternative to NHS ester of shortest spacer
- Amine reactive pegylation biotinylation reagent with a dPEG<sup>®</sup><sub>4</sub> spacer arm
- Optimal for solid phase synthesis, as alternative to the NHS ester (10200)
- Very water soluble, hydrophilic and eliminates non-specific binding upon labeling.
- Ideal spacer length for binding streptavidin conjugates; chain length from amide to terminal carbonyl is 19.2 Angstroms. Designed to be the same length as the LC-LC spacer, which is considered an ideal length for the streptavidin binding pocket.
- Non-antigenic and non-immunogenic spacer arm
- Application: ELIMINATES aggregation and precipitation when labeling antibodies and other biological materials (a severe problem with conventional biotinylation reagents)

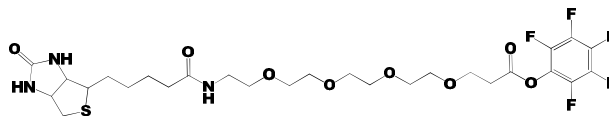
## Protocol:

See Hermanson (reference), pg. 728 for the NHS-dPEG<sup>®</sup><sub>4</sub> biotin. While the NHS ester can be used directly in water, this should, as the protocol calls, be dissolved in an organic solvent for optimal and easiest application.

## Reference:

Greg T. Hermanson, Bioconjugate Techniques, 2<sup>nd</sup> Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). Specifically see pp. 726-729 in his Chapter 18 on discrete PEG compounds.

Product #	Description	50 mg	1000 mg
10010	Biotin-dPEG <sup>®</sup> <sub>4</sub> -PFP ester	\$80	\$650



Mol. Wt.: 657.65; single compound  
dPEG<sup>®</sup> Spacer is 16 atoms and 19.2 Å



## Product Features and Benefits:

- CLEAVABLE
- Cleave point away from binding pocket for optimal cleavage
- Amine reactive CLEAVABLE biotinylation reagent with pegylation spacer for more effective binding, availability and solubility
- Disulfide is cleavable; replaces an inferior sulfo-NHS-S-S-biotin without the pegylation arm.
- Very water soluble, hydrophilic and eliminates non-specific binding.
- Ideal dPEG<sup>®</sup> pegylation spacer length for binding streptavidin conjugates; chain length from amide to terminal carbonyl is 28.7 Angstroms and 24 atoms. dPEG<sup>®</sup> linker is designed to be the same length as the LC-LC spacer, which is ideal for the streptavidin binding pocket.
- Non-antigenic and non-immunogenic pegylation spacer arm
- ELIMINATES aggregation and precipitation when labeling antibodies and other biological materials (a severe problem with conventional biotinylation reagents)

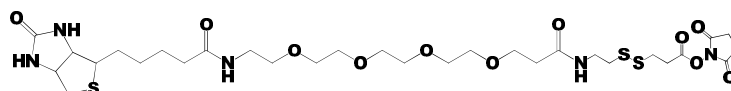
## Protocol:

See Hermanson (reference), pg. 728 for the NHS-dPEG<sup>®</sup><sub>4</sub> biotin. While this pegylation reagent can be used directly in water, this should, as the protocol calls, be dissolved in an organic solvent for optimal and easiest application

## Reference:

Greg T. Hermanson, Bioconjugate Techniques, 2nd Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). Specifically see pp. 726-729 in his Chapter 18 on discrete PEG compounds for pegylation applications.

Product #	Description	50 mg	500 mg
10194	NHS-S-S-dPEG <sup>®</sup> <sub>4</sub> -biotin	\$175	\$875



Mol. Wt.: 751.94; single compound  
dPEG<sup>®</sup> Spacer is 24 atoms and 28.7 Å



## Product Features and Benefits:

- Provides a second binding biotin for even stronger binding
- Amine reactive bis biotinylation pegylation reagent with a dPEG<sup>®</sup> spacer arm with potential to polymerize avidin or streptavidin at the label site.
- Very water soluble, hydrophilic and eliminates non-specific binding.
- Ideal spacer length for binding streptavidin conjugates total dPEG<sup>®</sup> length short enough to bend just one streptavidin subunit.
- Non-antigenic and non-immunogenic spacer arm
- Also ELIMINATES aggregation and precipitation when labeling antibodies and other biological materials (a severe problem with conventional biotinylation reagents)

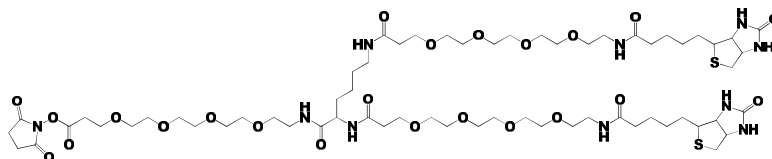
## Protocol:

See Hermanson (reference), pg. 728 for the NHS-dPEG<sup>®</sup><sub>4</sub> biotin. While this pegylation reagent can be used directly in water, this should, as the protocol calls, be dissolved in an organic solvent for optimal and easiest application

## Reference:

Greg T. Hermanson, Bioconjugate Techniques, 2<sup>nd</sup> Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). Specifically see pp. 726-729 in his Chapter 18 on discrete PEG compounds for pegylation applications.

Product #	Description	100 mg	1000 mg
10360	NHS-dPEG <sup>®</sup> <sub>4</sub> -Lys-(dPEG <sup>®</sup> <sub>4</sub> -biotin) <sub>2</sub>	\$150	\$1125



Mol. Wt.: 1437.72; single compound  
dPEG<sup>®</sup> Spacers are 40 and 35 atoms and 43.4 and 41.7 Å, resp.



## Product Features and Benefits:

- For biotinylations where serum samples are being used... Biotinidase is ubiquitous in serum and will rapidly cleave the biotin from detection system
- **Amine reactive biotinylation pegylation reagent** with a dPEG<sup>®</sup><sub>4</sub> spacer... perfect length for most applications.
- **BIOTINIDASE RESISTANT**... vital especially for in vivo work or clinical analysis.
- Very water soluble, hydrophilic and eliminates non-specific binding.
- This reagent gives the spacer length with the pegylation arm for optimal biotin binding with streptavidin conjugates; chain length from amide to terminal carbonyl is 19.2 Angstroms. Designed to be the same length as the LC-LC spacer, which is ideal for the streptavidin binding pocket.
- Non-antigenic, non-immunogenic and water soluble pegylation spacer arm
- ELIMINATES non-specific binding issues, things like aggregation and precipitation when labeling antibodies and other biological materials (a severe problem with conventional biotinylation reagents).
- **EXPECT GREAT, even dramatic S/N increases!!**

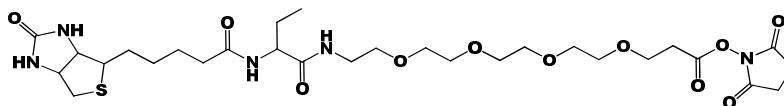
## Protocol:

The pegylation reagent can be pre-dissolved in an organic solvent OR can be directly dissolved in pure water, but must be used immediately. The reaction is best run between pH 7.2 and 8 using a non-amine buffer, e.g., PBS pH 7.2. Typical incubation times will be about 2 hours on ice and 30 minutes at RT. For large molecules the unreacted biotinylation reagent can be removed with gel filtration or dialysis.

## References:

D. Scott Wilbur, et al., "Biotin Reagents for Antibody Pretargeting. 7. Investigation of Chemically Inert Biotinidase Blocking Functionalities for Synthetic Utility," *Bioconjugate Chemistry*, 17(6), 1514-1522 (2006), and references therein. Plus Scott's earlier papers in this area: a) *Bioconjugate Chemistry*, 12, 616-623 (2001) and b) *Bioconjugate Chemistry*, 8, 572-584 (1997).

Product #	Description	100 mg	1000 mg
10202	NHS-dPEG <sup>®</sup> <sub>4</sub> -biotinidase resistant biotin	\$150	\$1200



Mol. Wt.: 673.78; single compound  
dPEG<sup>®</sup> Spacer is 19 atoms and 21.5 Å



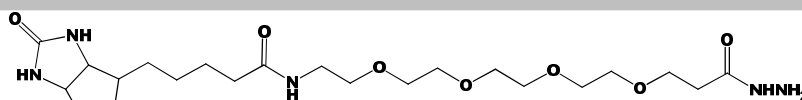
## Product Features and Benefits:

- Reacts beautifully with carbonyls, e.g., with aldehydes (reduced carbohydrates) without resorting to reductive amination
- Carbonyl reactive biotinylation reagent with a dPEG<sup>®</sup><sub>4</sub> pegylation spacer
- Reacts with aldehydes and ketones to give stable hydrazones in a single step.
- Also reacts with activated carboxylic acids
- Physical properties are similar to those of the NHS-dPEG<sup>®</sup><sub>4</sub>-biotin, helping to prevent or eliminate aggregation when labeling large biological, and products shows less non-specific binding than conventionally biotinylated compounds
- 20.6 Angstrom pegylation spacer arm (length from amide carbonyl to formed hydrazone N)
- Solubility in water: >50% w/v!

## Protocols: Ref.:

Greg T. Hermanson, Bioconjugate Techniques, 2nd Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). See pp. 733-736 for a general discussion of the chemistry and a general protocol for labeling of a glycoprotein is given on pg. 736!

Product #	Description	50 mg	1000 mg
10219	Biotin-dPEG <sup>®</sup> <sub>4</sub> -hydrazide	\$250	\$1100



Mol. Wt.: 505.63; single compound  
dPEG<sup>®</sup> Spacer is 18 atoms and 20.6 Å

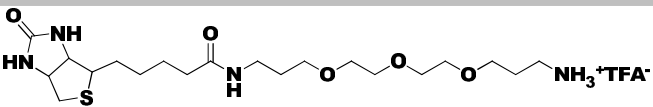
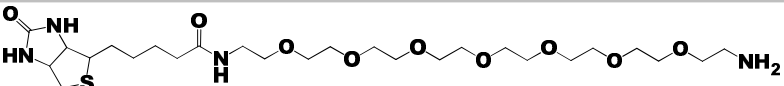
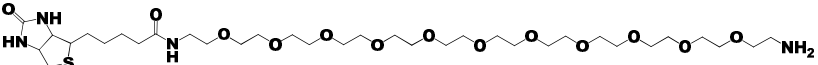
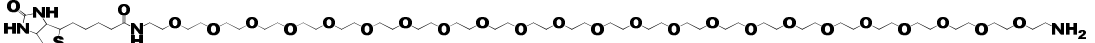


## Product Features and Benefits:

- x = 3, 7, 11 or 23
- Reacts with NHS or other active esters, or aldehyde or ketone in a reductive alkylation to 2° amine
- A Carbonyl reactive dPEG<sup>®</sup> pegylation biotinylation reagent, making the labeled product very hydrophilic and the biotin very available for streptavidin binding in the capture/binding step. With conventional reagents with the LC linker the biotin may lay on the surface of the labeled material and not be as available for binding streptavidin!
- Spacer is approximately the same length as 10200 (dPEG<sup>®</sup><sub>4</sub> with NHS ester), and its length is optimal for rapid and tight avidin/SA binding properties.

## Reference and protocol:

Greg T. Hermanson, Bioconjugate Techniques, 2<sup>nd</sup> Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). See pages 529 and 530 for a general discussion in Greg's book and on biotinylation as well, as well as pp. 737 and 738 for a protocol for a compound with just two oxygen. However, other than enhanced solubility and binding distances, the protocol is adaptable to our PN 10193.

Product #	Description	100 mg	1000 mg
10193	Biotin-dPEG <sup>®</sup> <sub>3</sub> -NH <sub>3</sub> <sup>+</sup> TFA <sup>-</sup>	\$100	\$750
 <p>Mol. Wt.: 560.63; single compound dPEG<sup>®</sup> Spacer is 15 atoms and 18.1 Å</p>			
10826	Biotin-dPEG <sup>®</sup> <sub>7</sub> -NH <sub>2</sub>	\$250	\$900
 <p>Mol. Wt.: 594.76; single compound dPEG<sup>®</sup> Spacer is 25 atoms and 29.8 Å</p>			
10196	Biotin-dPEG <sup>®</sup> <sub>11</sub> -NH <sub>2</sub>	\$275	\$1000
 <p>Mol. Wt.: 770.97; single compound dPEG<sup>®</sup> Spacer is 37 atoms and 44.1 Å</p>			
10786	Biotin-dPEG <sup>®</sup> <sub>23</sub> -NH <sub>2</sub>	\$300	\$1100
 <p>Mol. Wt.: 1299.60; single compound dPEG<sup>®</sup> Spacer is 71 atoms and 87.0 Å</p>			



## Product Features and Benefits:

- x = 3, 11 or 23
- Thiol reactive biotin with variable length spacers.
- Sulfhydryl reactive biotinylation reagent with dPEG<sup>®</sup><sub>x</sub> pegylation spacers!
- ONLY commercially available sulfhydryl reactive biotinylation reagents with hydrophilic spacer and maleimide functionality.
- dPEG<sup>®</sup> pegylation spacers provide HUGE practical advantages. Conventional thiol reactive biotinylation reagents are far too hydrophobic to be of practical commercial use; causing inherently insurmountable problems with solubility, aggregation and precipitation of biotinylated species. Many of these disadvantages have not been apparent due to the lack of alternatives. . . UNTIL NOW!
- Advantages: The dPEG<sup>®</sup> pegylation spacer makes the reagent water soluble, and the remarkable properties of the dPEG<sup>®</sup> aids in eliminating non-specific binding problem in the application due to the biotin and spacer combination, increasing S/N. These are often insurmountable barriers with conventional biotinylation reagents; spacer allows the vital biotin to stick out into the aqueous environment, and makes it more available than conventional reagents to binding with Streptavidin.

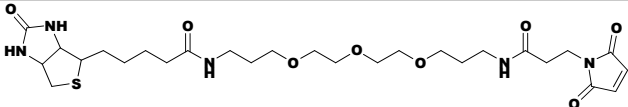
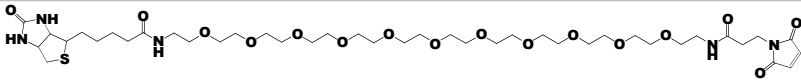
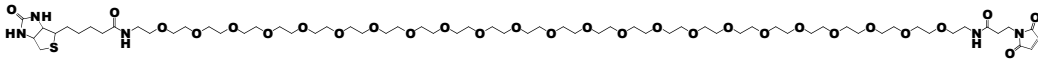
- Biotinylates at acidic to neutral pH, due to the incredibly high reactivity of the maleimide with thiols/sulfhydryls.
- Longer spacer may allow biotin accessibility of the biotin by the streptavidin/avidin conjugate binding system where the reactive thiols are buried in the labeled system, away from the surface..
- Pegylation arm is non-antigenic and non-immunogenic
- In solution there are no aggregation or precipitation problems when labeling antibodies and other biological materials (a known problem with conventional biotinylation reagents)

## Applications:

Biotinylation of a) sulfhydryl containing peptides and proteins; b) terminal sulfhydryl modified DNA

## Protocols & References:

Greg T. Hermanson, Bioconjugate Techniques, 2nd Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). See pp. 732-733 in Greg's new edition for a summary of the reactions, plus a typical protocol.

Product #	Description	25 mg	50 mg	100 mg
10201	Biotin-dPEG <sup>®</sup> <sub>3</sub> -MAL  Mol. Wt.: 597.73; single compound dPEG <sup>®</sup> Spacer is 21 atoms and 24.9 Å	NA	\$175	NA
10195	Biotin-dPEG <sup>®</sup> <sub>11</sub> -MAL  Mol. Wt.: 922.09; single compound dPEG <sup>®</sup> Spacer is 43 atoms and 50.5 Å	\$225	NA	\$450
10785	Biotin-dPEG <sup>®</sup> <sub>23</sub> -MAL  Mol. Wt.: 1450.72; single compound dPEG <sup>®</sup> Spacer is 77 atoms and 94.1 Å	\$250	NA	\$500

# Biotin-dPEG<sup>®</sup><sub>3</sub>-benzophenone



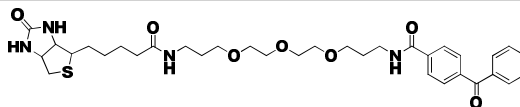
## Product Features and Benefits:

- Specific for abstractable hydrogens, e.g. photolabeling Carbohydrates, DNA, etc.
- Can be used in water
- Finally, an AFFORDABLE PHOTOREACTIVE biotinylation reagent for photoaffinity labeling with a pegylation spacer
- Contains a hydrophilic, non-immunogenic dPEG<sup>®</sup> pegylation spacer arm
- The additional advantages of benzophenone as a photoactivating group include the following:
  - Does not react with an aqueous solvent medium when activated with light
  - Activated with wavelengths least damaging to proteins (>300 nm)
  - Stable in ambient light
  - Gives non-specific insertion into C-H bonds in a variety of biologically relevant targets, especially HO-C-H
  - Activation is non-photodissociative

## References:

- Excellent reviews for the general use of benzophenone containing compounds:
- G. Dorman and G. D. Prestwich, "Using Photolabile Ligands in Drug Discovery and Development," Trends in Biotechnology, 18, 64-77 (2000).
- G. D. Prestwich, et al., "Benzophenone Photoprobes for Phosphoinositides, Peptides and Drugs," Photochemistry and Photobiology, 65(2), 222-34 (1997).
- G. D. Prestwich, et. al., "Benzophenone Photophores in Biochemistry," Biochemistry, 33(18), 5661-5673 (1993).

Product #	Description	25 mg	100 mg
10267	Biotin-dPEG <sup>®</sup> <sub>3</sub> -benzophenone	\$125	\$275



Mol. Wt.: 654.82; single compound  
dPEG<sup>®</sup> Spacer is 15 atoms and 16.9 Å

# Biotin-dPEG<sup>®</sup><sub>3</sub>-TFPA

## A Non-specific dPEG<sup>®</sup> Photoaffinity Label



### Product Features and Benefits:

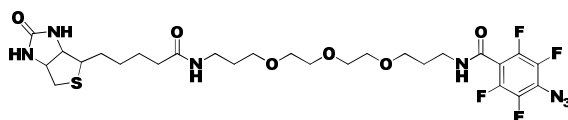
- FIRST dPEG<sup>®</sup> non-specific photoaffinity reagent
- will react with just about anything!
- TRULY NON-SPECIFIC PHOTOREACTIVE biotinylation reagent for non-specific labeling of ANY organic containing compounds, surfaces or particles. It has the perfect spacer arm that contains a hydrophilic, non-immunogenic dPEG<sup>®</sup> pegylation spacer arm
- Also, the dPEG<sup>®</sup> pegylation spacer will reduce or eliminate issue of aggregation and non-specific binding (noise) typical of convention LC spacers...it is PERFECT!!
- Advantages of tetrafluorophenyl azide as a photoactivating group include the following:
  - Reacts with C-H and N-H bonds non-specifically from the photogenerated singlet nitrene with high efficiencies.
  - Highly efficient incorporation
- Historical perspective: It has been claimed for many, many years that the commercially available phenyl azides are non-specific. This is INCORRECT!! They are not only very, very inefficient, but are also completely specific for amines. The perfluorophenyl azides have ideal photophysical properties, and have been designed to overcome the deficiencies in these old reagents.

### References:

Excellent references for the tetrafluorophenyl azides include the following:

- Chemistry and Kinetics of Singlet (Pentafluorophenyl)nitrene, M. S. Platz, et al., J. Amer. Chem. Soc., 111B4, 5054-5067 (1992).
- Synthesis and Binding of New Polyfluorinated Aryl Azides to alpha-Chymotrypsin. New Reagents for Photoaffinity Labeling. M. S. Platz, et al., Bioconjugate Chemistry, 4,256-261 (1993).
- Synthesis of a Tetrafluoro-Substituted Aryl Azide and Its Protio Analogue as Photoaffinity Labeling Reagents for the Estrogen Receptor, J.A. Katzenellenbogen, et al., J. Org. Chem., 56, 3125-3133 (1991).
- NHS Ester Functionalized Perfluorophenyl Azides as Novel Photoactive Heterobifunctional Cross-linking Reagents. J.F.W. Keana, et al., Bioconjugate Chemistry, 5, 151-157 (1994).

Product #	Description	25 mg	100 mg
10308	Biotin-dPEG <sup>®</sup> <sub>3</sub> -TFPA	\$125	\$275



Mol. Wt.: 663.69; single compound  
dPEG<sup>®</sup> Spacer is 15 atoms and 16.9 Å

# Biotinoylsarcosine



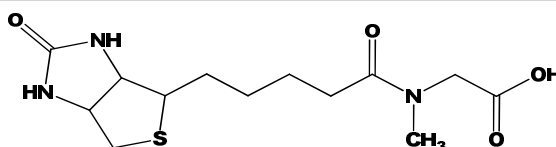
## Product Features and Benefits:

- Building block for **BIOTINIDASE RESISTANT** biotinylation reagents
- Inquire about the NHS ester
- The sarcosine is one spacer that has been developed by Dr. Scott Wilbur at the University of Washington to give derived biotinylation reagents a high level of biotinidase resistance, while maintaining a high binding rate and affinity for the avidin and streptavidin

## References:

D. Scott Wilbur, et al., "Biotin Reagents for Antibody Pretargeting. 7. Investigation of Chemically Inert Biotinidase Blocking Functionalities for Synthetic Utility," *Bioconjugate Chemistry*, **17(6)**, 1514-1522 (2006), and references therein. Plus Scott's earlier papers in this area: a) *ibid.*, **12**, 616-623 (2001) and b) *ibid.*, **8**, 572-584 (1997).

Product #	Description	1000 mg
10355	Biotinoylsarcosine	\$250



Mol. Wt.: 315.39; single compound

# Biotinoyl-2-Aminobutyric acid



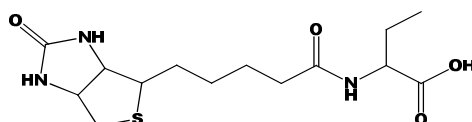
## Product Features and Benefits:

- Building block for **BIOTINIDASE RESISTANT** biotinylation reagents
- Inquire about the NHS ester
- The 2-aminobutyric acid is another yet simple and effective spacer that has been developed by Dr. Scott Wilbur at the University of Washington to give derived biotinylation reagents a high level of biotinidase resistance, while maintaining a high binding rate and affinity for the avidin and streptavidin

## Reference:

D. Scott Wilbur, et al., "Biotin Reagents for Antibody Pretargeting. 7. Investigation of Chemically Inert Biotinidase Blocking Functionalities for Synthetic Utility," *Bioconjugate Chemistry*, **17(6)**, 1514-1522 (2006), and references therein. Plus Scott's earlier papers in this area: a) *Bioconjugate Chemistry*, **12**, 616-623 (2001) and b) *Bioconjugate Chemistry*, **8**, 572-584 (1997)

Product #	Description	1000 mg
10356	Biotinoyl-2-Aminobutyric acid	\$250



Mol. Wt.: 329.42; single compound



## Product Features and Benefits:

- x = 7, 11, 23 or 47
- Click on biotin
- Completely unique dPEG<sup>®</sup>- containing biotinylation pegylation reagent containing terminal azide functionality, which can be used as a protected amine, or available as a partner in either a click or Staudinger coupling application. The azide can be reacted with an acetylene moiety (Click reaction) or an arylphospine as part of several Staudinger ligation options (see references)
- dPEG<sup>®</sup> spacer is extremely hydrophilic and non-immunogenic/ non-antigenic
- Spacer will reduce or eliminate problems with aggregation and immunogenicity -- typically issues with conventional crosslinkers

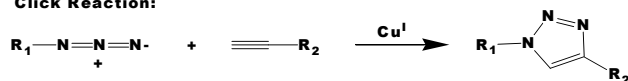
## Applications:

Two very active areas of development using the azide functionality are a) "Click" chemistry, the particular example of the Cu(I) catalyzed reaction of the azide and a terminal acetylene; and b) the Staudinger ligation using functionalized aryl phosphines to couple the azide in a covalent fashion to form amides.

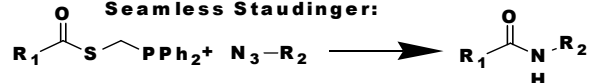
The tremendous attraction to the azide functionality is its very low reactivity and high stability under most conditions, especially where other conjugating functionality have to be used very cautiously due to their limited stability, or require careful control of variables like pH in order to insure high yielding reactions. However, under very specific conditions, the azide is very reactive and highly selective in its reactivity.

As is the case in the current very economically competitive environment, many of the applications of these chemistries may be protected intellectual property.

### Click Reaction:



### Seamless Staudinger:



## Protocols:

For particular protocols, please look in the references cited or more detailed application references contained within.

## References:

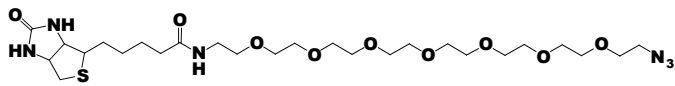
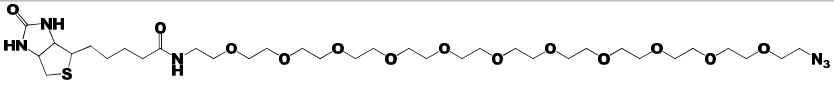
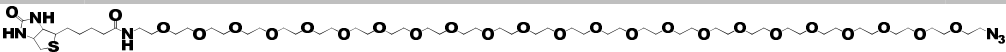
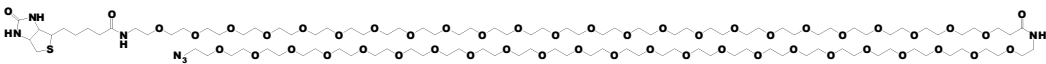
**General:** See Greg T. Hermanson, *Bioconjugate Techniques*, 2<sup>nd</sup> Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0), and specifically see pp. 722-724.

**Click Applications:** a. "Click Chemistry: Diverse Chemical Function from a Few Good Reactions," H. C. Kolb, M.G. Finn, and K. Barry Sharpless, *Angew. Chem., Int. Eng. Ed.*, 40, 2004-2021 (2001); b. "The growing impact of click chemistry on drug discovery," H. C. Kolb and K. Barry Sharpless, *Drug Discovery Today*, 8(24), 128-137 (2003); c. "Cu(I)-Catalyzed Alkyne-Azide "Click" Cycloadditions from a Mechanistic and Synthetic Perspective," V. C. Bock, H. Hiemstra and J. H. van Maarseveen, *Eur. J. Org. Chem.*, 51-68 (2006); d. "A Rapid and Versatile Method to Label Receptor Ligands Using "Click" Chemistry: Validation with the Muscarinic M1 Antagonist Pirenzepine," *Bioconjugate Chemistry*, 17, 1618-1623 (2006).

**Staudinger ligations:** a. "The Staudinger Ligation-A Gift to Chemical Biology," M. Kohn and R. Breinbauer, *Angew. Chem. Int. Ed.*, 43, 3106 (2004); b. "Traceless Staudinger Ligation of Glycosyl Azides with Triaryl Phosphines: Stereoselective Synthesis of Glycosyl Amides," A. Bianchi and A. Bernardi, *J. Org. Chem.*, 71, 4565-4577 (2006); c. "Reaction Mechanism and Kinetics of the Traceless Staudinger Ligation," M. Soelner, B. L. Nilsson and R. T. Raines, *J. Amer. Chem. Soc.*, 128 (27), 8820-8828 (2006). The first reference is an excellent and recent review in a very active area. Search engines for "Staudinger ligation" for many excellent and additional references.

# Biotin-dPEG<sup>®</sup><sub>x</sub>-azide (cont.)



Product #	Description	100 mg	1000 mg
10825	Biotin-dPEG <sup>®</sup> <sub>7</sub> -azide	\$200	\$1350
 <p>Mol. Wt.: 620.32; single compound dPEG<sup>®</sup> Spacer is 27 atoms and 30.7 Å</p>			
10784	Biotin-dPEG <sup>®</sup> <sub>11</sub> -azide	\$250	\$1400
 <p>Mol. Wt.: 796.97; single compound dPEG<sup>®</sup> Spacer is 40 atoms and 50.4 Å</p>			
10787	Biotin-dPEG <sup>®</sup> <sub>23</sub> -azide	\$300	\$1600
 <p>Mol. Wt.: 1325.60; single compound dPEG<sup>®</sup> Spacer is 73 atoms and 87.7 Å</p>			
10780	Biotin-dPEG <sup>®</sup> <sub>47</sub> -azide	\$350	\$1800
 <p>Mol. Wt.: 2453.94; single compound dPEG<sup>®</sup> Spacer is 154 atoms and 186.5 Å</p>			



## Product Features and Benefits:

- Biotin building block
- Amine reactive label
- Quanta's exclusive synthetic process produces the highest quality commercial material
- Efficient label for certain peptides and nucleic acids
- Permeates cell membranes

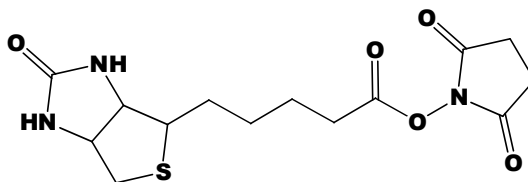
## Protocol:

Hermanson, pg. 512, except make a 40 mg/ml solution of the NHS-biotin in dry DMF. The use of dry DMF is critical. This can easily be obtained by placing 3 Angstrom or 4 Angstrom molecular sieves into reagent grade DMF, shaking and letting stand at least overnight. If possible handle the NHS-biotin under an inert atmosphere. We find that handling these materials in an Atmosbag, available from Sigma-Aldrich, under argon (preferred) or nitrogen, is the best, most convenient and inexpensive means of handling these moisture sensitive materials. The remaining solid NHS-biotin should be stored under an inert gas (argon preferred) at about 4°C.

## General Biotin/Biotinylation References:

- Wilchek and Bayer: "Avidin-Biotin Technology", Methods in Enzymology, Vol. 184, M. Wilchek and E. A. Bayer, eds., 1990, Academic Press, NY.
- Hermanson: Greg T. Hermanson, Bioconjugate Techniques, 2nd Ed, Elsevier Inc., Burlington, MA 01803, April, 2008 (ISBN-13: 978-0-12-370501-3; ISBN-10: 0-12-370501-0). . Note: This reference is an extremely practical and informative volume from an experienced practitioner.

Product #	Description	100 mg	1000 mg
10205	NHS-biotin	\$50	\$150



Mol. Wt.: 341.38; single compound

# Bis-dPEG<sup>®</sup><sub>3</sub>-biotin



## Product Features and Benefits:

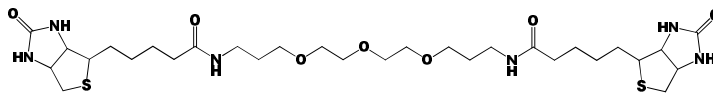
- Unique crosslinking reagent for avidin and streptavidin
- Contains a dPEG<sup>®</sup><sub>3</sub> pegylation spacer in order to enhance the practical use of the reagent in aqueous systems.

D. Scott Wilbur, et. al., "Biotin Reagents for Antibody Pretargeting. 2. Synthesis and in Vitro Evaluation of Biotin Dimers and Trimers for Cross-Linking of Streptavidin" *Bioconjugate Chemistry*, **11**, 819-832 (1997)

## References

D. Scott Wilbur, et. al., "Biotin Reagents for Antibody Pretargeting. 3. Synthesis Radioiodination, and Evaluation of Biotinylated Starburst Dendrimers" *Bioconjugate Chemistry*, **11**, 813-825 (1998)

Product #	Description	50 mg	1000 mg
10325	Bis-dPEG <sup>®</sup> <sub>3</sub> -biotin	\$100	\$750



Mol. Wt.: 672.90; single compound  
dPEG<sup>®</sup> Spacer is 15 atoms and 18.1 Å

# Biotin-dPEG<sup>®</sup><sub>3</sub>-cyanocobalamin



## Product Features and Benefits:

- Quantitate biotin binding
- Biotin reagent for analytical measurements of biotin binding to streptavidin for other biotinylated species. The reagent has been uniquely designed as a competitive standard reagent to assess the relative binding of biotin derivatives with avidin and streptavidin (on/off rate determinations)
- No other comparable reagent is available anywhere else.
- dPEG<sup>®</sup> reagent contains a 3 unit pegylation chain for optimal binding distance and physical properties to balance to incredible hydrophilicity of biotin.
- Designed by Dr. Scott Wilbur at the University of Washington (see references)

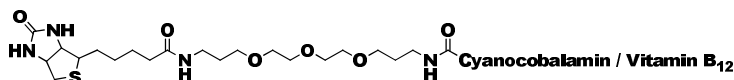
## References:

- D. Scott Wilbur, et. al., "Biotin Reagents for Antibody Pretargeting. 4. Selection of Biotin Conjugates for in Vivo Application Based on Their Dissociation Rate from Avidin and Streptavidin," *Bioconjugate Chemistry*, 11, 569-583 (2000)
- D. Scott Wilbur, et. al., Evaluation of Biotin-Dye conjugates for Use in an HPLC Assay To Assess Relative Binding of Biotin Derivatives with Avidin and Streptavidin," *Bioconjugate Chemistry*, 11, 584-598 (2000).

## Applications and protocols:

- Please refer to the following publications by Scott Wilbur for details on using this reagent.

Product #	Description	5 mg
10218	Biotin-dPEG <sup>®</sup> <sub>3</sub> -cyanocobalamin	\$125



Mol. Wt.: 473.61; single compound  
dPEG<sup>®</sup> Spacer is 15 atoms and 18.1 Å