NT-PEGYLu

PEGylation reagents (linkers, crosslinkers and labels)

What is pegylation ?

Pegylation is the chemical modification of any compound or material by PEG agents (an acronym of PolyEthylenGlycol), that includes a carbone/oxygen backbone and displays unique properties to the modified compound (or conjugate, surface), starting with improved hydrophilicity. Pegylation thus has become a widespread technique to confers hydrophilic properties to any substances or material, such as labels, drugs, or surfaces, but also to increase the molecular weight, to create polymeric structures or gels (hydrogels). They also allow to introduce functional groups or haptens that add their own properties. As a result, pegylation is used in many areas, from biomedical and pharmaceutical applications (probes, biocaptors, drugs, vaccines,…), to industry like cosmetics (ex gels, emoliants, pow,…), materials or optoelectronics (coating, paints, detectors,…).

This technical notice presents main pegylation methods and their useful PEG reagents:

- Pegylation basis / fundamentals:
  What is pegylation? ; PEO versus PEG, dPEG,… and other kinds;
  Problems in pegylation ; PEG properties
- Pegylation of biomolecules by target functional groups:
  Pegylation of Amines, Carboxyls, Hydroxyls, Thiols
- Pegylation of surfaces : Gold, Glass and silicones, other tips
- Special pegylation approaches:
  Pegylation of N-terminal and C-term amino acids of peptides; Click-type methods, Branched pegylation;
  Reticulation; Hydrogels; …
- PEG application domains:
  Cosmetics | New improved materials | Medecine & Pharma industry (Drug development)

Overview of available PEG/PEO reagents & search on-line:

Type/spacer :		
PEG (from 160-950Da to 1-300KD)	PEO (from 2 to 48)	Branched (multi-arms): arm (from 2 to 9)
Functional groups - XLink		
mPEG(methoxyl-PEG, methyl-PEG) NH2(Amine), COOH(Carboxyl), OH(hydroxyl), SH(Sufhydryl) NHS(Succinimidyl), MALeimide, N3(Azide), HYDrazide, ALKyne, DBCO, TCO, Tetrazine, Acrylate, Epoxy, OPD(OrthoPyridylDiSulfide), MTS		,

Functional groups - XLink		
Cholesterol, Folate, Biotin, Lipoic acid		

*Pegylation basis / fundamentals

Pegylating a molecule or a support consists to graft chemically* (or conjugate) it with hydrophilic chains of PEG/PEO, in order to change the physicochemical properties at the molecular level (hydrophilicity, Molecular Weight, steric volume or hindrance…) and at macroscopic levels (physically, biochemically and/or biologically).

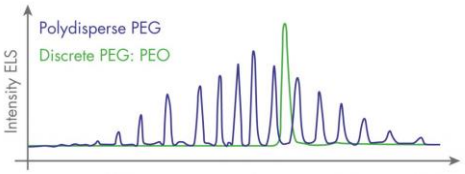
See the article 'La PEGylation: definition, principes, méthodes et avantages'[R06]:

- What is PEG

-Polydisperse length and MW

- What is PEO (or dPEG)

-Definite length (PEO units) and MW



- Properties of Polyethylene Glycol
- Properties conferred to conjugates
- Applications

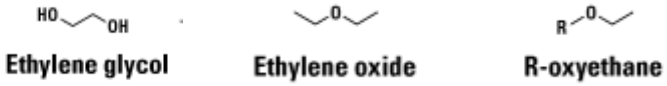
PEG/PEO/dPEG:[]

PEG (acronym for PolyEthyleneGlycol) is a generic term for polymeric glylcol based compounds, that cover different kinds of products depending on manufacturers. Some are purified, other are synthetized. These products display a *variety of spacer lengths* around the given length (or molecular weight). This **dispersivity** is often 5 to 10% for *purified materials*. Even with lower values, this dispersivity can generate variations that can become critical after conjugation for final applications.

We, at Interchim, use the term **PEOn** (for n units of PolyEthylOxy motif) for those PEG compounds that have precise PEG length and MW (a definite number or PEO units). They are obtained by state-of-art chemical synthesis. They are also known as dPEG.

See how you can trust Uptima to address such problems and limitations.

PEG versus PEO, PEO, PEG, dPEG ?



All terms correspond basically to the same chemical structure, and refer to repeats of ethylene glycol units. Depending on how one chooses to define the constituent monomer or parent molecule, on may then call:

PEG is the common abbreviation for PolyEthylene Glycol – or, more properly, poly (ethylene glycol), EG units)) –.

PEO refers to 'PolyEthylene Oxide' (also referred as **POE** for 'PolyOxyEthylene')

TEG for Tetra EthyleneGlycol or Tri-Ethylne Glycol, but also DEG for Di-Ethylene Glycol, PEG for Penta-Ethylene Glycol, **HEG** for Hexa-Ethylene Glycol, …

NB: in fact, the term PEG is often employed for 2 different configurations, depending on both ends (A and B): a PolyEG motif is typically . A-[CH_2CH_2-O]$_n$-B . (PolyEthyleneOxy)

but sometimes refers to . A'-[O-CH_2-CH_2-]$_x$-B' . (PolyOxyEthylene)

(the 2 are identical only when, for example, A=OH and B=H (and A'=H and B'=OH)

Due to important benefits and rising use in labs, from diagnostics to therapeutics, the term **PEGylation** has been introduced to refer any use of this important class of reagents, typically when one covalently attach or modify surfaces, proteins and other molecule.

However, the simple PEG term can be misleading… Quality/price of these PEG compounds differ greatly depending there are synthetized(single compounds) or, more classically, made from purified PEG (polydisperse sizes): most available commercially PEG are mixtures of different oligomer sizes which size and molecular weight (MW) span a range that is more or less broad/narrow. For example, "PEG 10000" product will have an average MW of 10 000 g/mol (usually defined without the added functional groups) and the number of PEG repeats can range from n = 195 to n=215, or elsewhere from 170 to 255 ! Such **polydispersity**, that is even amplified upon further reactions, introduce various behaviors in downstream applications (hydrophilicity, mobility, stability,...). Polydispersity complexifies greatly the interpretation of results in applications such as pharmacology (kinetics and distribution), or bioassays (kinetics and sensitivity). For these applications, **monodisperse** PEG/PEO compounds are greatly recommended (obtained by full synthetic methods).

● Interchim use the term '**PEG reagents**' for compounds that are **oligodisperse** (have a number of EG units and the length span typically ±<10% around a mean value – they are obtaining by strictly controlled purification,so CV is often rather ±2-5%. They are named PEG_x MW nnnnDa, or PEG^{nnnn} , where x indicates that the given MW is a mean value, eventually indicated in place of x as nnnn in Daltons (the spacer molecular weight), ranging from 200-400-600-800 (Da) and from 1000 (1 000Da, or 1KDa) to 40000 (40 000Da, or 40KDa).

● **PEO_n** refers at Interchim more specifically to monodisperse compounds (n units ranging from 2 to 96. Equivallent to **dPEG** (for 'discrete PEG'). PEO_n designates that the PEG compound has a perfectly defined PEO/PEG structure (n = unique number of ethylene glycol / ethylene oxide units), hence has an accurate length and expectable conformation. State of art synthesis allows to polymerize 6, 12 or 24 units, and even up to 96 units as pure compounds. They can also be designated as **PEG_{nn}**, where nn is the number of EO units, ranging typically from 2 to 48.

Number of units and PEG lengths (PEOn/PEGnn and POEGxxxx)

calculates n (the nb of units) from xxxx (average molecularWeight) and vice-versa:

Use formula: . $MW_{peg} = n \times 44$.

PEO_n (PEG_{nn})	#units	MW	PEG-MW* PEGxxx	$\#_{approx}$units (x=$n_{av.}$)	$_{approx}$MW	Commentary et sources
	n=1	MW=**44**	(EO, OE)		MW=**44**	Unit (monomer)
	n=2	MW=62.07	EthyleneGlycol			
PEO4	**n=4**	176	PEG-**200**	x=~4.5 (n=3to6)	~200	minimal PEO length available .
PEO8	**n=8**	352	PEG-**350**	x=~7.95	~350	
PEO9 ($dPEG_9$)	**n=9**	396	PEG-**400** (PEG_9)	x=~9.09	~400	
PEO48 ($dPEG_{48}$)	n= 48	2112	PEG-**2000** (PEG_{48})	x=~45.45	~2 000	maximal PEO length available.
-	n= 228	10032	PEG-**10000**, PEG**10K**	x=~227.3 (n=195 to 265)	~10 000	
-			PEG-**30000**, PEG**30K**	x=~682	~30 000	Usual max PEG length available
-			PEG-**300K**	x=~6818	~300 000	Largest PEGs

*Note that the given MW does not account for termini groups, whatever they are H (+2), OH (+16), Methyl (mPEG) or other functional group (reactive groups such as NHSuc, or labels such as Biotin).

Other PEGs kinds

In (bio)chemistry labs, for biotechnology, PEG generally designates PolyEthylenOxy-polymers. This applies to Uptima PEGs, unless stated otherwise.

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However in industry, other kinds of PEG are more popular:

The 2 terminal primary hydroxyl groups of the basic PEGs can create **mono-, di- and poly-esters, amines, ethers and acetals**. Furthermore, PEGs can create additional compounds and complexes through a reaction in their ether bridges. Overall, PEG derivatives may include **PEG ethers** (e.g. laureths, ceteths, ceteareths, oleths, and PEG ethers of glyceryl cocoates), **PEG fatty acids** (e.g. PEG laurates, dilaurates, stearates, and distearates), **PEG castor oils**, **PEG amine ethers** (PEG cocamines), **PEG propylene glycols**, and other derivates (e.g., PEG soy sterols and PEG beeswax).

PEGs properties[]

PolyEthyleneGlycol (PEG) (or PolyEthylOxy: PEO) structure improves features of your conjugates compared to conventional spacers (i.e. alkyls based) thanks to their hydrophilicity, flexibility, andadjustable spacer length.

PEG/PEO technology benefits :

- Increases water-solubility
- **Minimizes aggregation** of conjugates or conjugates/ligands complexes
- Not immunogenic
- **Increases bio-stability**
- choice of the spacer length
- **Reduces non-specific bindings** on surfaces or samples
- choice of functional groups at termini

Physical properties of Polyethylene Oxy / Polyethylene Glycol

The **molecular weight** of PEGs is determined by the number of ethylene glycol units incorporated into each PEG polymer and vary from 300 grams per mole to 10 000 000 grams per mole. Mean MW value and dispersivity of purified PEG is addressed above.

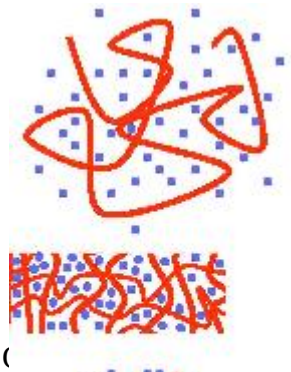
Low molecular weight PEGs, containing two-to-four ethylene glycol units per polymer, are **clear, watery liquids**.

PEGs containing up to 700 ethylene glycol units per polymeric product are **clear, thick liquids**.

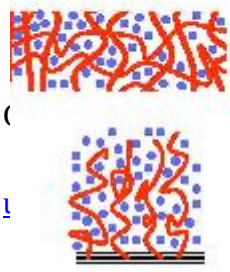
PEGS having 1 000 or more ethylene glycol units per polymeric product are **waxy solids**.

PEGS are odorless, colorless, nonirritating and do not evaporate easily. PEGs are considered inert (they do not react with other materials), and they are non-toxic. PEGs are soluble in many organic solvents. All PEGs readily dissolve in water and do not change the color, odor or taste of the water.

Biotech Properties of PEGs []



Poly(ethylene glycol) unique properties make it unique features (a) free in solution, (b) cross-linked, and (c) grafted to a surfaces, for example, end-tethering. It is especially useful in various biological, chemical and pharmaceutical settings: PEO/PEG technology increases reagent and conjugate solubility, minimize toxic and immunological effects compared to non-PEO/PEG spacers, and provide several options for accommodating specific crosslinking distances.



● **Hydrophilic (aqueous-soluble)** – PEO/PEG inherent hydrophilicity is not only conferred to the reagent, allowing solubility in aqueous buffer (avoiding the use of organic solvents, often hazardous), but also conferred to the modified molecule or surface. As a result, PEO/PEG conjugates have increased solubility and are more bio-stable and bio-compatible: less prone to aggregation, less immunogenic (no antibodies raised again the spacer), more bio-available in organism, …

● **Spacing effect (adjustable lengths) and flexibility of PEG chain** – provides less steric hindrance. As a result, compared with alkyl- and furthermore aryl-spacers, higher kinetics are achieved for the conjugates that should bind with ligand partners in aqueous buffers. PEO/PEG treated surfaces are more available for polar reactions. Background is reduced in detection systems.

● **Non-toxic and non-immunogenic** – PEO/PEG by itself does not interfere with cellular functions or target immunogenicity. This applies to in its original form (excess reagent) or after conjugation (to a surfaces and an other biomolecule). In pharma applications, bioavailability is increased.

● **Flexibility in fonctionalization** – PEO/PEG spacer can be activated with quite any functional groups (reactive groups, bulky groups, labels, ligands...), at its termini or inside. As a result, PEGylation is **compatible with any chemistry** of conjugation/labeling/functionalization

● **Adjustable lengths** – the length of PEO/PEG spacer can be set precisely from very short to extra-long. Such adjustable lengths are useful to fit requirements of many applications, depending on desired characteristics of surfaces, ligands or probes, conjugate MW, hydrophilicity, length... Hydrophilic gels as well can be done with adjusted porosity.

Advantages of Pegylation : conferred properties

Pegylation agents are polar, hence **soluble** in aqueous solutions that render them suitable for biological/biochemical applications. **Superior concentrations** can be performed during the conjugation step, allowing higher coupling ratios.

More interestingly, the hydrosolubility is carried by the spacing arm (and not by an additional polar group, such as sulfoNHS that is removed after coupling), hence the hydrophilicity is held by the formed conjugate:

- The hydrophilic PEG chains favorize the **solubility of conjugates** in aqueous buffers. This reduce **aggregation** that is sometimes observed using classical crosslinkers. This also avoids precipitations and **increases the stability** of the conjugate;
- The **hydrophilicity** of a pegylated drug changes its **distribution** between compartments in cells and organism: it will generally be less sequestrated in fatty compartments and more **bioavailable**.
  The **motility** is increased in a aqueous solution, modifying in general favorably the **interaction** of a drug or affine probes toward its target (enzyme, receptor on a biological membrane,…)
- The **molecular weight and size** of a conjugate are increased, and can by modulated by the length of the PEO/PEG chain. Small PEG grafting can increase hydrosolubility with a limited growth in size. At the opposite, a drug pegylated by large PEG will not by filtrated by kidneys.
- The steric volume, notably **hydrodynamic volume**, is also increased. This allows to **protect** bioactive compounds against a proteolytic activity, change the **properties of surface** of a support and its interactions with solutes or other supports (anti **aggregate** effect for particles).
  Very interesting macromolecular properties appear : absorption capacity and viscosity of **hydrogels** ; **sequestration/release** of active agents, **amphipilicity** of polymer alternating hydrophilic and hydrophobic motifs, CMC and aggregation number of **micelles**).
- The pegylation agents can be **branched. T**hat allows to create original structures (ex. **dendrons**) in solution or immobilized. The pegylation then generates singular properties at the physical (through stericity), chemical and even biochemical (through multivalency) levels.

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- Finally, PEGylation agents are available **fonctionnalized** by any groups used in classical chemistries (NHS/Amine, Maleimide/Sulfhydryl,…) and moderns (click chemistry). It is possible to graft labels (fluorophore, tag,…), affine groups, substrates, antigens, oligos or peptides, in a bioorthogonal manner (without interference with biological compounds / even in vivo). The conjugation can associate several functions, same or different, that combine or interfere in the PEG polymer or with other partners (AntibodyDrugConjugate, affinity + substrate, bispecific binding, FRET, molecular beacons,).

The many possible structures of PEGS build **great molecular scaffold**, and their combination, also to other components, make **conjugates designs quite infinite**!

Problems in PEGylation:

Problems encountered during pegylation works may come from: a)the PEG compounds features and quality (manufacturing), b)improper use (conjugation chemistry; ratio of coupling; .

a> Problems due to PEG compounds quality / manufacturing:

[]

1)**polydispersivity** in PEG lenght

Uptima PEG reagent addresses this concern at 2 levels, by providing PEG products clearly designated as 2 grades (see above §):

- PEG products named PEGx are purified (disperse) compounds, and their polydispersivity is <10% (this can be documented on the certificated of analysis.

- PEG products named PEO_n (or PEG_{nn}) are synthetic (monodisperse) compounds, so devoid of different MW compounds (or as trace - documented on the certificate of analysis)

2)**diol PEG**: even in a mPEG, a certain amount of PEGdiol is present, in the range of 1-10%, depending on the purification quality and on the molecular weight (lower or low-mass PEG). These diol compound appears as a pic or shoulder in front of the main elution peak by GPC (MW twice of the monofunctional mPEG). High diol concentration will yield unwanted cross-linked conjugates.

Uptima PEG reagent address this concern by high quality purification shema and QC. Undesired compounds or as trace (ddocumente on the certificate of analysis)

3)**undesired branched PEG**

Again Uptima addresses this concern by high quality purification schema and QC for his PEG reagents.

Please feel free to contact Uptima expertise for these concerns or others in your demanding application.

b> Problems dues to used conjugation method.

The PEGylation (the conjugation of PEG spacer) may be not specific or have insufficient yield because of the used conjugation chemistry method. Have in mind that any chemical reaction is not strictly specific and total, although some are much more specific and give nice yield in usual conditions, like click chemistry.

For protein conjugation, Succinimides (NHS) and Maleimide are the most popular conjugation reagent because they are well specific, respectively to amine and sulfhydryls, once proper conditions of reaction (notably buffer type, pH) are used, despite competition with water yield are acceptable / can be managed using higher coupling ration of reagent/to/protein.

But the click reactions are surely the more remarkable reactions to get highest specificity (including in complex biological samples: biorthogonality). Reaction and coupling yields are also generally good, using proper condition and sometimes catalyzer (Cu2+ in the classic click reaction CuAAC; Aniline in the HyNic Hydrazone chemistry). The ultimate reaction yield goes to Azocycloaddition.

Finally, the specificity and yield of the reaction may be balanced by stability issues:
For example the iodoacetamide reaction is less specific than maleimide, but gives more stable link! [XLfctl]

Please feel free to contact Uptima expertise for these concerns or others in your demanding application.

*Pegylation of target functional groups

The following gives helpful hints about the PEGylation (the conjugation of PEG spacer) of a variety of classical target chemical groups fond in biomolecules.

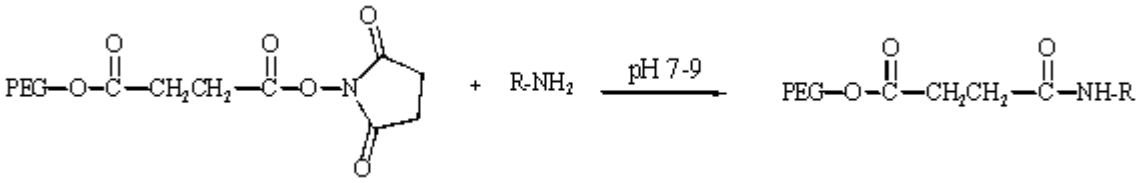
AMINE PEGylation

Amine group is an ubiquitous functional group found in any protein and some other biomolecules. It is also introduced in modified nucleotides during PCR synthesis using aminoallyl NTPs for site directed coupling; and also for metabolic labeling. Amines are convenient target groups for bioconjugation because they are nucleophilic leading to many useful reactive reactions.

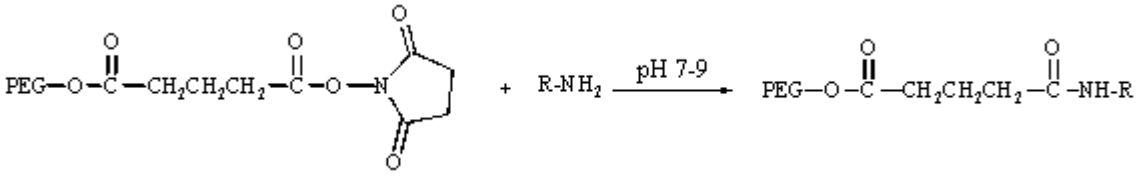
Amine can be coupled by reaction of different kinds of esters (Succinimides (SC, SCp, SPA, SVA, SS, SG) | Imido-Esters | Phenyl-Esters (STP, PFP)), of nitrophenyl carbonate, of IsothioCyanate, and of aldehydes.

More information about these reactions[]

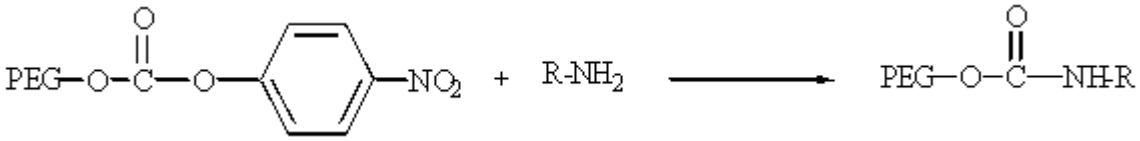
- **PEG-SS** (Succinimidyl Succinate) pegylates amine groups under mild conditions by forming amide bond. The ester linkage in the backbone is susceptible to hydrolytic cleavage.



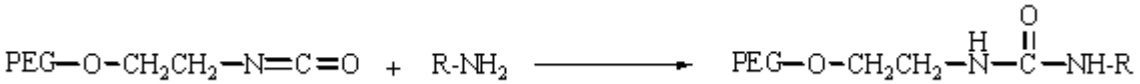
- **PEG-SG** (Succinimidyl Glutarate) pegylates amine groups of the target compound. The PEG-SG may be more resistant to hydrolysis than the PEG-SS because of an additional CH2 chain.



- **PEG-NPC** (p-nitrophenyl carbonate) is also reactive toward amines, and forms a stable urethane linkage.



- **PEG-isocyanate** is also a useful derivative for pegylation of amine groups resulting in a stable urethane linkage.



- **PEG-aldehyde** may pegylate amines through sodium cyanoborohydride reduction. The reaction pH may be important for target selectivity, and N-terminal amine pegylation may be at around pH 5.

- mPEG-Propionaldehyde Please inquire <>
- mPEG-amide-Propionaldehyde Please inquire <>

NT-PEGYLu

- mPEG-urethane-Propionaldehyde Please inquire <>
- mPEG-Butylaldehyde Please inquire <>

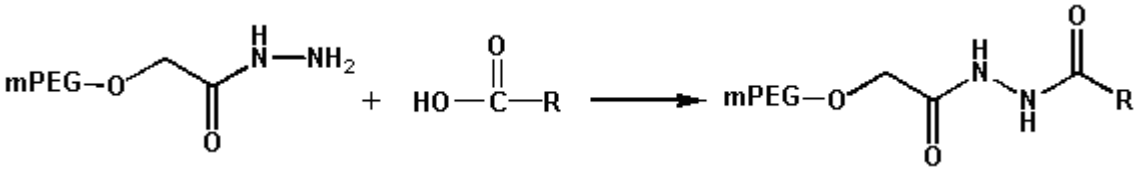
CARBOXYL Pegylation

Carboxyl groups can be coupled by reactions of an Hydrazide.

More information about these reactions[]

•**PEG-hydrazide** can pegylate carboxyl group quite selectively in presence of N,N'-dicyclohexylcarbodiimide (DCC), or in presence of a water soluble coupling agent such as N-(-3dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

The very low pKa (~3) of the hydrazide group allows carboxyl pegylation in acidic media in presence of EDC. The carboxyl groups of a protein which are readily activated with EDC at mild acidic pH, tend to react readily with PEG-hydrazide, whereas amino groups of the protein remain non-active.



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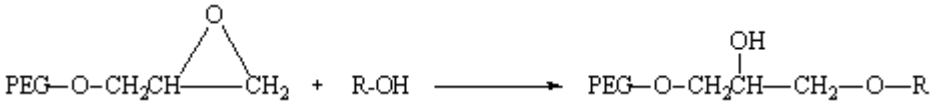
HYDROXYL PEGylation

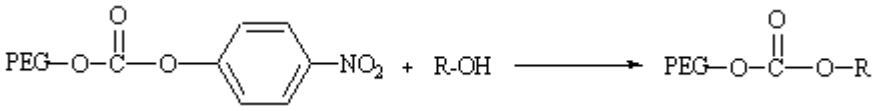
Hydroxyls can be pegylated by reaction of Epoxides, NPC, IsoCyanate

More information about these reactions[]

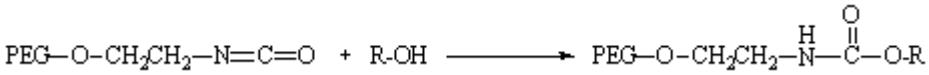
PEG-epoxide pegylates hydroxyl groups at a mild reactivity and is most effectively coupled at higher pH(8.5-9.5). The PEG-epoxide may also pegylate hydoxyl, amine, thiol groups. The PEG-epoxide shows excellent water stability at basic pH.



•**PEG-NPC** also pegylates hydroxyl groups



•**PEG-isocyanate** also pegylates hydroxyl groups yielding stable urethane linkage. However, its reactivity may be best exploited for pegylating non-peptide moieties such as drugs, hydroxyl-containing matrices for chromatography, and also to produce biocompatible surfaces.

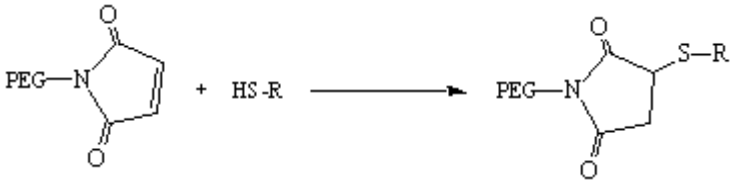


THIOL PEGylation

The **Thiol** group (SH, Sulfhydryl) is a interesting functional group for bioconjugation because it is present in many proteins, and it can be targetted relatively specifically by conventional chemistry (disctintively for the amine groups, also nucleophilic).SH can be coupled by reaction of maleimides, halogeno alkyls (Iodo/Bromo, haloacetyl/haloacetamides), Thiosulfinates (ThioSulfonates &VinylSulfone). It also binds to other thiol group by exchange to form a disulfide bridge (Pyridyl thiols).

More information about these reactions[]

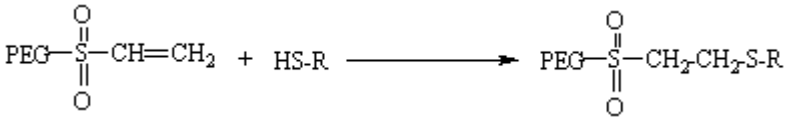
•**PEG-maleimide** pegylates thiols of the target compound in which the double bond of the maleimic ring breaks to connect with the thiol. The rate of reaction is pH dependent and best conditions are found around pH 8. But when the pH is higher than 8 and organic co-solvents are present, it has been known cause a side-reaction in which PEG-maleimide may react with amines, although this side-reaction is significantly slower.



More information about these reactions[]

Search maleimides PEG reagents; Thiol PEG [] See Thiol PEGreagents

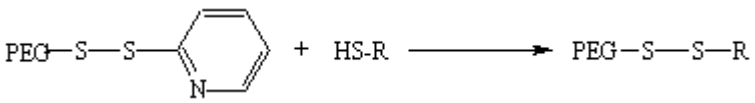
•**PEG-vinylsulfone** pegylates thiols of the target compound in the same way as in PEG-maleimide.



More information about these reactions[]

Search VinlySulfone PEG reagents (Vinyl;…)

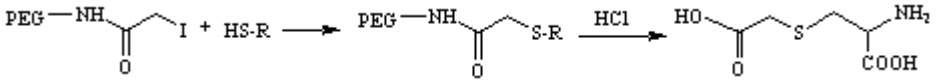
•**PEG-orthopyridyl-disulfide (OPSS)** also pegylates thiols resulting in disulfide bond. The conjugation can be decoupled by treatment with reductase such as $NaBH_4$.



More information about these reactions[]

Search disulfides PEG reagents (PyridylDiThio;…)

•**PEG-iodoacetamide** pegylates thiols to form stable thioether bonds in mild basic media. This type of conjugation presents an interesting aspect in that by strong acid analysis the pegylated cysteine residue of the protein can give rise to carboxymethylcysteine which can be evaluated by a standard amino acid analysis (for example, amino acid sequencing), thus offering a method to verify the occurrence of the reaction.



More information about these reactions[]

Search HaloAlkyl PEG reagents: Bromo/Iodo , acetyl/acetamides

•**PEG-ThioSulfonate** .

More information about these reactions[]

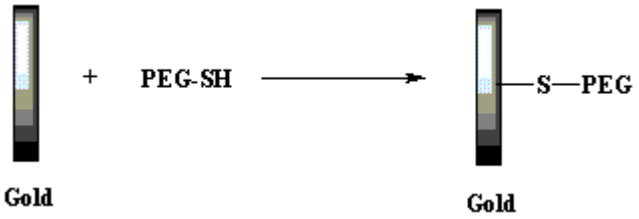
Search ThioSulfonate reagents: MTS;…

Applications of PEG-Thiols: •metal surface binding: cf below.

*Pegylation of surfaces

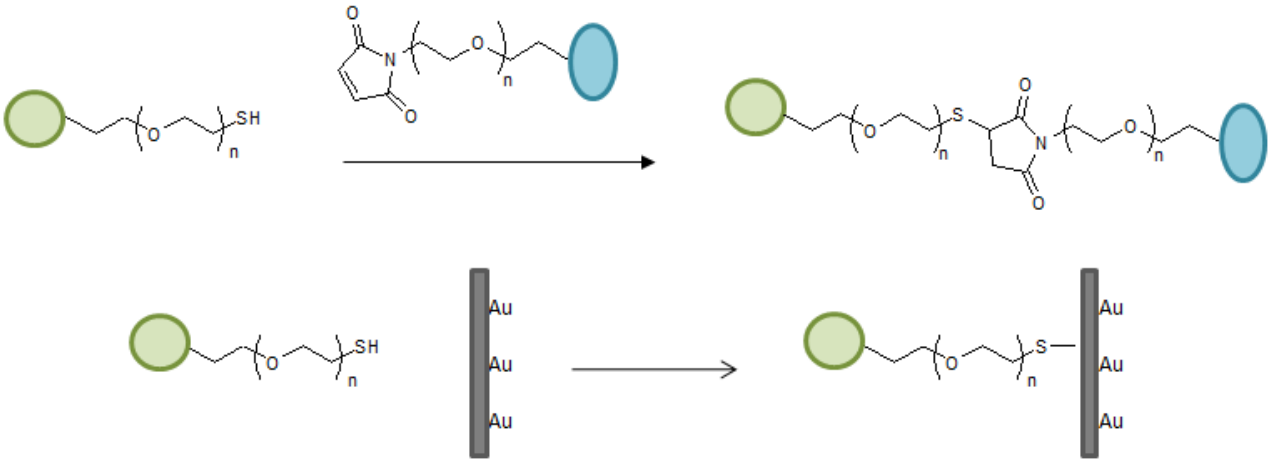
METAL SURFACE PEGylation

•**PEG-thiol** pegylates gold surface with high level of specificity.



The gold-thiol reaction may be viewed as an oxidative addition of the S-H bond to the gold, followed by a reductive elimination of the hydrogen.

$R\text{-}S\text{-}H + Au^{o} \text{ ----> } R\text{-}S^{-}Au^{+}Au^{o} + 1/2H_2$



[].

GLASS/Silicone PEG-fonctionalization

Silane (Si) functionalized PEG (PEG-Si), can be used to modify glass, silica, silicone and other surfaces via the reaction between hydroxyl group and triethoxyl silane. They can thus functionalize these support with groups that will confer PEG's physico-chemical properties, such a amine, COOH, or SH, Maleimide, succinimidyl (NHS), and Biotin. Hence

-pegylation can greatly suppress the non-specific binding of charged molecules to the modified surfaces.

-one can prepare 'activated' supports that can be further treated (conjugation, coating, SAM reagents), and used typically in detection systems such as biocaptors, microscopy slides, microarrays,... They have wide applications for medical

devices, biomedical/ biological microelectromechanical systems (bioMEMS) and biocompatible material development.

Other applications include special optical lenses, purification, ...

=>See Silane-PEG products[FT]

*Special pegylation methods

Click-pegylation

Click-type reactions involve 2 partnering groups that will react readily specifically (hence without interfering with other chemicals, notably as bond in biological materials -bioorthoganoality-.

Although these chemistries can be applied to pegylate biomolecules or materials, this approach is of few interest in most applications when most pegylation are performed in vitro.

However, pegylation is a great technique to introduce in biomolecules or on materials functional groups for further click chemistries (azide, alkyne, DBCO, BCN, Tetrazine,…): heterobifunctional PEG-crosslinkers containing one clickable group, are used for conjugation through the other of their group by standard chemistry as described above.

See the click chemistry technical notice[][NT-XLclic] (reactions and functional groups)

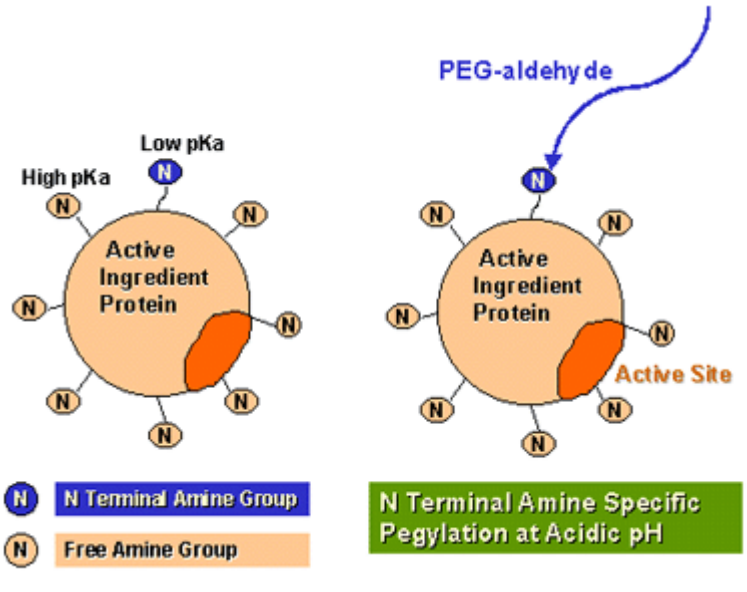
Search clickable PEG reagents [NT-XLclic]

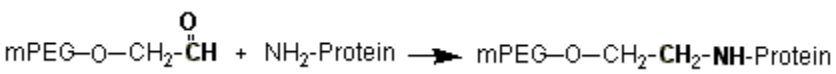
Pegylation of peptides at N- terminus

PEG can be attached to the N-terminal amino group of proteins and peptides. This N-terminal pegylation may allow advantage in purification of the conjugates. It can also better preserve bioactivity as compared to a random pegylation of amino group of lysine residues. The agents used in achieving the N-terminal specific Pegylation are PEG-aldehydes. This is facilitated by the difference in pKa values of the amino group of lysines(~10) and of amino group of N-terminal amino acid(~7.6- 8.0), which allows pH dependent nucleophilic attacks to the electrophilic PEG-aldehydes.

PEG-aldehyde: PEG-aldehyde may pegylate N-terminal amino group of peptides and proteins. The reaction pH is important for the N-terminal amine specific pegylation and the pH may be at around pH 5.

N-terminal pegylation may be conducted, for example, in 100 mM sodium acetate or 100 mM sodium biphosphate buffer at pH 5.0~6.0. The buffer may additionally contain 20 mM sodium cyanoborohydride. The molar ratio of compound to mPEG-aldehyde may be 1:5 ~ 1:10. The pegylation is then stirred overnight at ambient or refrigeration temperature.

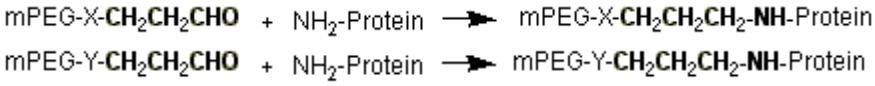




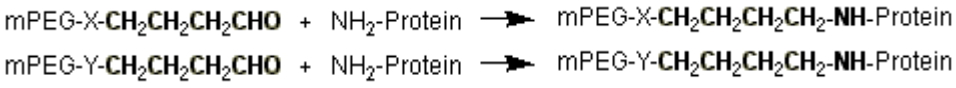
Rem: PEG-aldehyde also participate to click-like reaction with hydrazines: see CHO/HyNic Hydrazone chemistry[NT-XLclic].

See Aldehyde PEG reagents (Acetaldehyde, Propionaldehyde)

PEG-propionaldehyde: several novel PEG-propionaldehydes were developed to overcome the relatively nonspecific and weak N-terminal pegylation using PEG-aldehyde.



PEG-butylaldehyde: several novel PEG-butylaldehydes were developed that can also be useful for N-terminal pegylation.



Pegylation of peptides at C-terminus

PEG can be attached to the C-terminal carboxyl group of proteins and peptides, provided the sequence of the peptides does not contain COOH-containing aminoacids such as Glu, Asp (or that are bloqued).

A conventional method is an amidation between a PEG-Amine and the target COOH, typically activated (by a Sucinimidyl) and/or mediated by a carbodiimide (EDAC,…). See technical sheet of EDC (FT-52005A).

See Amine-PEGs.

Pegylation of peptides at Cys-terminus

A classic approach when synthetizing a peptide for further conjugation, it to include a Cys amino-acid at one of its terminus, generall the C-terminus. The availability of PEGs containing SH-specific reactive groups such as maleimide (see §-Thiol pegylation) allows easy pegylation of the peptide at its C-term, presenting the bioactive sequence usually located in the center of the peptide.

See Thiol-PEGs.

PEGs and Hydrogels – for cell culture, 3D Bioprinting,…

Hydrogels consist of a hydrophilic macromolecular network that forms a gel in aqueous solutions. PEGs offer a remarkable core structure to make hydrogels, thanks to its core hydrophilicity. Furthermore, branched PEGs are great building blocks, and can arbor functional groups for further polymerization or to add specific active compounds or properties (hormones, drugs, dielectric, chelates, inks,…).

Hydrogels are applied as media support for 3D cell culture and innovative possibilities in tissue and organ engineering, or for other applications such as **3D printing**. Multi-arm PEG and PEG-PCL acrylate and methacrylate derivatives are useful for such hydrogels.

See Branched PEGs

Ask for PEG-PCL

Branched pegylation

PEG are basically linear polymeric compounds. Branched structure can be created using multivallent terminus between linear PEGs, However an easier and more powerfull approach is to use branched PEGs (branching usually occurs at a carbon atom of the PEO unit: tri-, tetra-, hexa-, octa-vallent) with functional groups (or Methyl-blocking group) displayed at termini. Advanced branched PEGS include dendrimers. PEG with intermediate functional group(s) can be created also. See Branched PEGs section.

These branched PEGs are useful for preparing compounds or materials that are multivalent, high mlolecular mass/sized, reticulated. Examples include hydrogels, vaccines, dendrimeric particles and dendrons, …

Other Pegylation tips

In progress

+

*PEG reagents by functionality (and structure)

See an highlight on PEO/PEG/TEG reagents[BB013c] for detailed descriptions of PEG reagents for organic synthesis, modification of proteins, labeling:

- mPEG (Methyl, MethylOxy)
- Amine-reactive PEG Reagents
- Carboxyl-reactive PEG Reagents
- Sulfhydryl-reactive PEG Reagents
- Clickable-reactive PEG Reagents
- Linkers for organic synthesis

…

mPEG:

(in progress)

Thiol PEG reagents

The thiol PEG reagents are reactive with maleimides, disulfides, haloacetamides, and other thiols. It can also be used for metal surface binding (see Surface pegylation).

See Mono-functional PEGs (&Homo-Bi-)

See Hetero-bi-functionnal PEG / Silane PEG

Maleimides PEG reagents

(in progress)

Disulfides PEG reagents

(in progress)

Haloacetamides PEG reagents

(in progress)

Aldehyde PEG reagents (Acetaldehyde, Propionaldehyde, Butylaldehyde)

Aldehyde group reacts with amine groups at pH5-8, and is useful for the conjugation to N-terminal amine of peptides in the presence of a reducing reagent [Lorey 2014,YU 2018].

Propionaldehyde group is reative to amine groups but less selective and more reactive compared with acetaldehyde. It reacts at pH5-8, and is useful for the conjugation to N-terminal amine of peptides in the presence of a reducing reagent [Lorey 2014,YU 2018].

Butylaldehyde group is a latest possible group.

Aldehyde also participate to click-like reaction with hydrazines: see CHO/HyNic Hydrazone chemistry[NT-XLclic].

See Maleimide-PEGx-Aldehyde #AWK5X1 &all.
mPEG-Propionaldehydes, mPEG-Butylaldehydes
Inquire for other PEG-Aldehydes
See also peptides N-terminal Pegylation, C-terminal Pegylation

Lipidic PEGs

See hetero bifunctional PEG with lipid groups (DSEP, Folic Acid,…)

Clickable PEGs

Clickable PEG compounds include PEG and PEO spacers harboring functional groups that suit click chemistries, such as Azides and improved version, alkynes and improved versions.
More info in NT-Clickation

Other functionalitized PEGs:

Inquire.

*PEG reagents by structures

[]

Raw material: mPEG (PEG-O-CH3)

• Small sized PEGs (monodisperse: synthetic PEOs)

#03521 EG Ethylene Glycol MW: 62.07; CAS: 107-21-1 (Syn.: 2,2'-[Ethane-1,2-diylbis(oxy)]di(ethan-1-ol))

#01931 DEG Di-Ethylene Glycol MW:106.12; CAS: 111-46-6 (Syn.2,2'-Oxydi(ethan-1-ol)); Ethylene diglycol; Diglycol; 2,2'-Oxybisethanol; 2,2'-Oxydiethanol; 2,2'-Oxybis(ethan-1-ol); 3-Oxa-1,5-pentanediol; 2-(2-Hydroxyethoxy)ethan-1-ol; Dihydroxy diethyl ether)

#03072 TEG Tri-Ethylene Glycol MW: 150.17; CAS: 112-27-6 (Syn.: 2,2'-[Ethane-1,2-diylbis(oxy)]di(ethan-1-ol))

#03806 TtEG Tetra-Ethylene Glycol MW: 194.23; CAS: 112-60-7 (Syn.: Bis[2-(2-hydroxyethoxy)ethyl] ether, Tetraglycol)

#23822 PEG Penta-Ethylene Glycol MW: 238.28; CAS: 4792-15-8 HO-PEG_4^{or5}-OH HO-PEO_4-H

NT-PEGYLu

#23763 HEG Hexa-Ethylene Glycol MW: 282.33; CAS: 2615-15-8 HO-PEG$_5^{or6}$-OH HO-PEO$_5$-H

#BK922 OEG Octa-Ethylene Glycol MW: 370.44; CAS: 5117-19-1 HO-PEG$_6^{or7}$-OH HO-PEO$_6$-H

#BG248 HpEG Hepta-Ethylene Glycol MW: 326.19; CAS: 5617-32-3 HO-PEG$_7^{or8}$-OH HO-PEO$_7$-OH

#BK871 NEG Nona-Ethylene Glycol MW: 414.25; CAS: 3386-18-3 HO-PEG$_8^{or9}$-OH HO-PEO$_8$-H

#BK061 DEG Deca-Ethylene Glycol MW: 582.81; CAS: 5579-66-8 HO-PEG$_9^{or10}$-OH HO-PEO$_9$-H

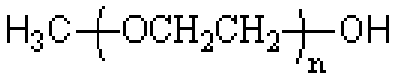
($C_{20}H_{42}O_{11}$; Syn.: Polyoxyethylene (10); 3,6,9,12,15,18,21,24,27-nonaoxanonacosane-1,29-diol)
(and less precisely: Polyethylene glycol 500, PEG500; and improperly: PEG-10)

• Small sized PEGs (polydisperse: purified)

FT-WU0992 (mPEG) Generic CAS # for PEGs ($C_{2n}H_{4n+2}O_{n+1}$): 25322-68-3

• MethylOxy-PEGs (mPEGs)

mPEG^x-OH (^{x}: any MW between PEG1K and PEG30K)

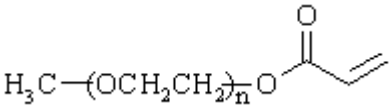


Mono-functionnal PEGs (&Homo-Bi-) derivatives

MonoFunctionnal PEG (mPEG-X)

Homo-Bi-Functional PEGs (X-PEG-X)

And more: acrylate, Amine, epoxide,…

	Molecular Weight	Product Name	Chemical Structure
	30K	mPEG-acrylate	
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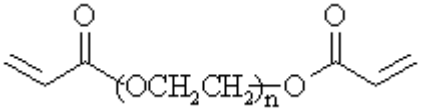
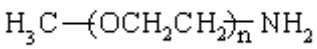
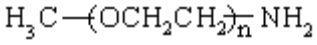
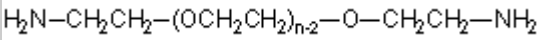
	12K		
	10K		
	5K	mPEG-acrylate	
	2K		
	10K	PEG-(acrylate)$_2$	
	8K		
	6K		
	3.4K		
	2K		
	30K	mPEG-amine	
	20K		

	12K		
	10K		
	5K	mPEG-amine	
	2K		
	10K	PEG-(amine)$_2$	
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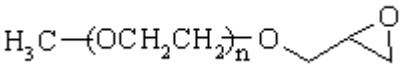
	20K	mPEG-epoxide	
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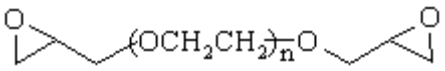
	10K		
	5K		
	2K		
	10K	PEG-(epoxide)$_2$	
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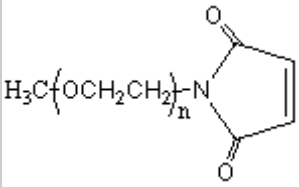
	6K		
	3.4K		
	2K		
	30K	mPEG-maleimide	
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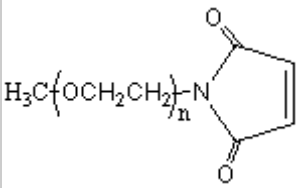
	5K	mPEG-maleimide	
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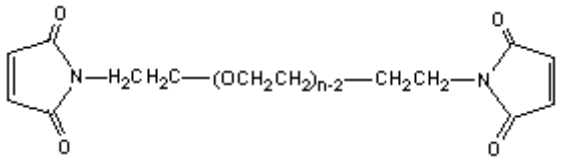
	10K	PEG-(maleimide)$_2$	
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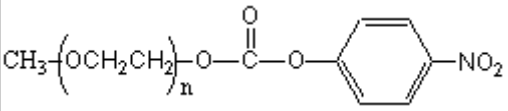
	30K	mPEG-nitrophenyl carbonate	
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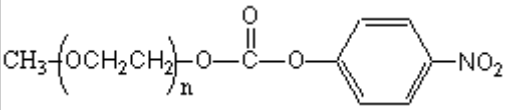
	10K		
	5K	mPEG-nitrophenyl carbonate	
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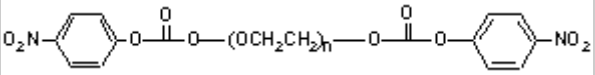
	10K	PEG-(nitrophenyl carbonate)$_2$	
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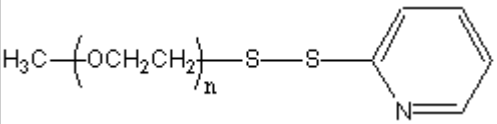
	2K		
	30K	mPEG-orthopyridyl disulfide	
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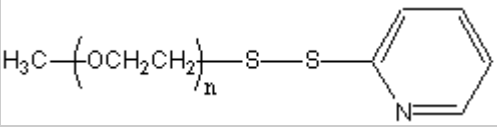
	5K	mPEG-orthopyridyl disulfide	
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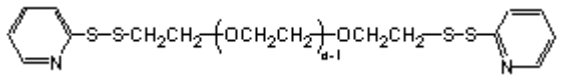
	10K	PEG-(orthopyridyl disulfide)$_2$	
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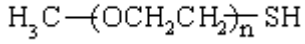
	30K	mPEG-sulfhydryl	
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	12K	mPEG-sulfhydryl	
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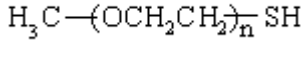
	5K	mPEG-sulfhydryl	
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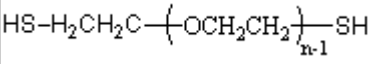
	10K	PEG-(sulfhydryl)$_2$	
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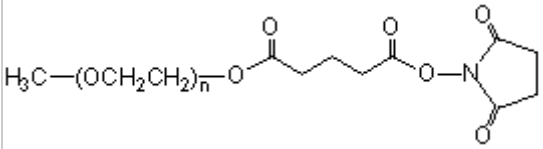
	2K		
	30K	mPEG-succinimidyl glutarate	
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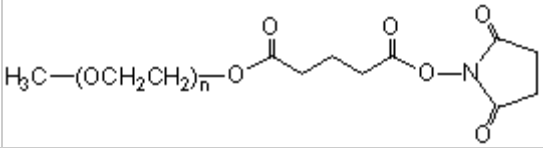
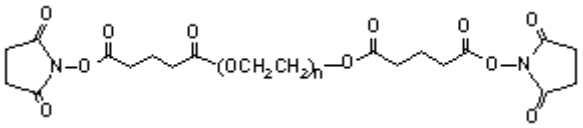
	10K		
	5K	mPEG-succinimidyl glutarate	
	2K		
	10K	PEG-(succinimidyl glutarate)$_2$	
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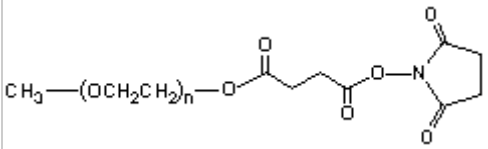
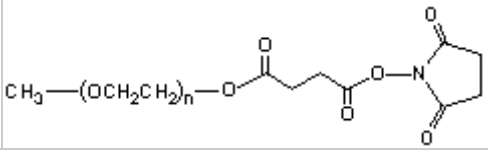
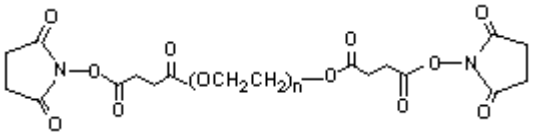
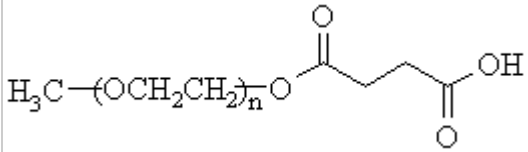
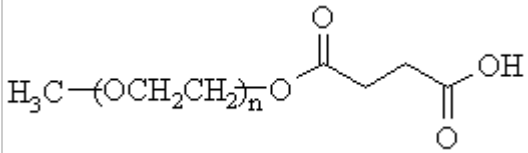
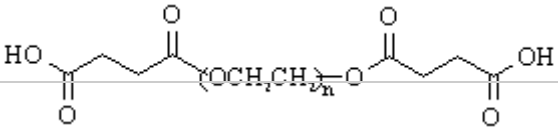
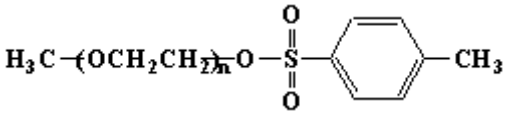
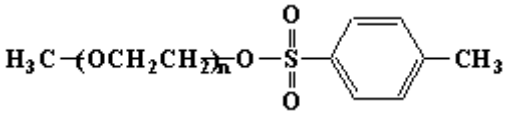
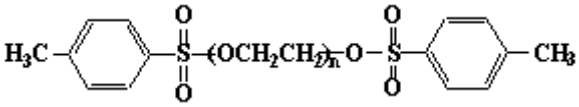
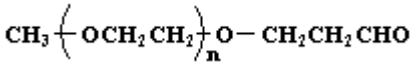
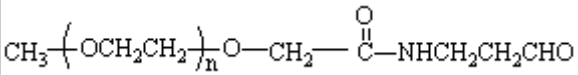
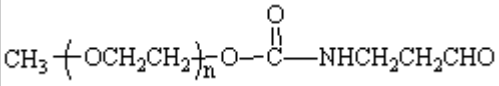
	6K		
	3.4K		
	2K		
	30K	mPEG-succinimidyl succinate	
	20K		
	12K		
	10K		
	5K	mPEG-succinimidyl succinate	
	2K		
	10K	PEG-(succinimidyl succinate)$_2$	
	8K		
	6K		
	3.4K		
	2K		
	30K	mPEG-succinic acid	
	20K		
	12K		
	10K		
	5K	mPEG-succinic acid	
	2K		
	10K	PEG-(succinic acid)$_2$	
	8K		

NT-PEGYLu

	6K		
	3.4K		
	2K		
	30K	mPEG-tosylate	
	20K		
	12K		
	10K		
	5K	mPEG-tosylate	
	2K		
	10K	PEG-(tosylate)$_2$	
	8K		
	6K		
	3.4K		
	2K		

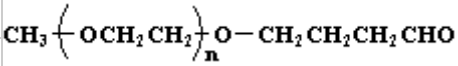
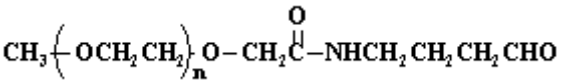
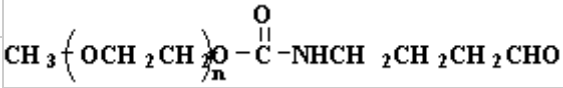
<*>

Unique mPEG-propionaldehydes

Product Name		M.W.	Chemical Structure
mPEG-propionaldehyde		5K	
		10K	
		20K	
		30K	
mPEG-amide-propionaldehyde		5K	
		10K	
		20K	
		30K	
mPEG-urethane-propionaldehyde		5K	

Unique mPEG-butylaldehydes

<*>

Product Name		M.W.	Chemical Structure
mPEG-butylaldehyde		5K	
		10K	
		20K	
		30K	
mPEG-amide-butylaldehyde		5K	
		10K	
		20K	
		30K	
mPEG-urethane-butylaldehyde		5K	

Branched PEGs (&Multi-arm, Y-shaped PEG, Forked PEG)

FT-WU0101 (MultiArm-Functional-PEGs)
FT-A2TY21 (4ARM-PEG-SCM&co)
FT-WU1191 (4Arms-PEG-NHSesters)
FT-LV5640 (Branched Maleimide PEOs)

Branched PEGs have particular conformational and/or branched (ramified) structures that display physical properties not possible with standard (linear) PEGs. They also are polymer of PEO units (or TEG, HEG,…), but the linkage of the oligomer are on some tetravalent carbons or heteroatoms (not C or O for the PEO unit).

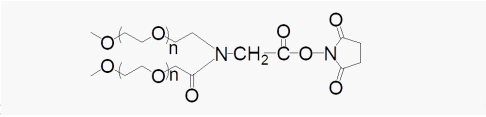
ON can distinguish branched PEGS by the type of branching (chemical link of 'nodes'), or by the number of branch (arms), and hyper-branched such as dendrimers that are of regular structure.

2-arms PEGs (Forked PEGs, Y-shaped PEGs)

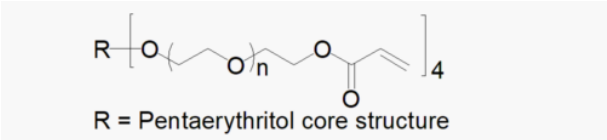
2-arm PEG, also known as **forked PEG**, display a structure that places two reactive groups at precise distance apart. They have become very popular for mimicking the heavy chain domain in an antibody or fragment antibody and other applications where two proteins in proximity are advantageous.

Exemple with []

2-arm PEGS can also display two linear methoxy PEG chains attached to a central core with one functionnal group. Such derivatives are also named **Y-shape PEG**. Their sterically bulky structure may help to reduce the number of attachment sites to a protein molecule.

Example with NHS group:  []

4-arm PEGs, **6-arm PEGs** and **8-arm PEGs** are available with several branching cores:

2arm PEG	See above
4arm PEG Acrylate  []	

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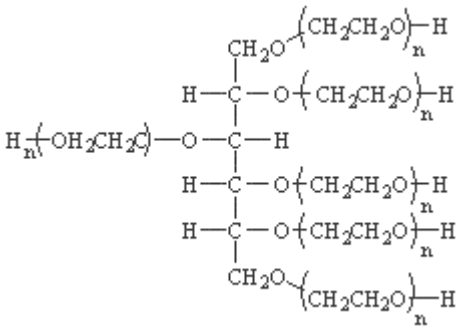
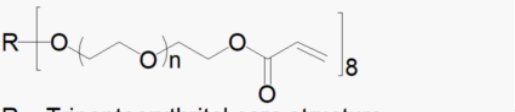
6arm PEG	
8arm PEG Acrylate (hexaglycerol) []	8arm PEG Acrylate (tripentaerythritol)  []

See 2-arm PEGs | 4-arm PEGs | 6-arm PEGs |
8-arm PEGs (pentaerythritol core) | 8-arm PEGs (hexaglycerol core.)

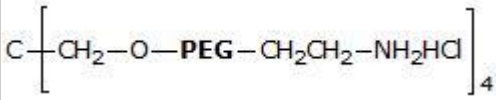
+

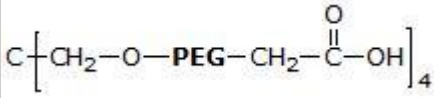
4-arm PEGs (| back to other branched PEGs

FT-A2TY21 (4ARM-PEG-SCM&co)

And more[]:

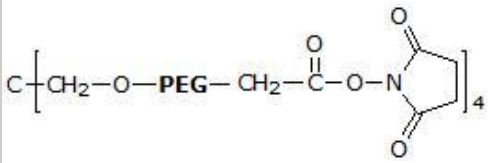
*()

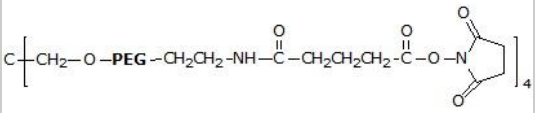
4-Arm PEG-Amine  See below for other MW (13-15-17K)	2K	1J282A
	5K	WU110A
	10K	WU111A
	20K	WU112A
	40K	1J285A

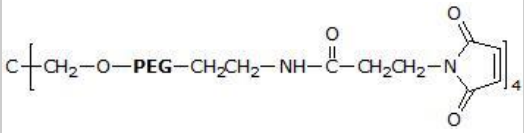
4-Arm PEG-COOH 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

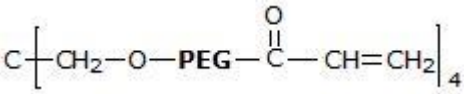
4-Arm PEG-SCM (Succinimidyl Carboxymethyl Ester)	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire

NT-PEGYLu

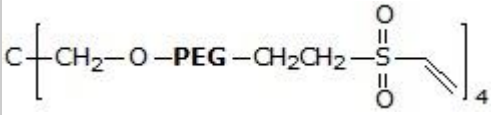
		
	40K	Inquire

4-Arm PEG-SGA (Succinimidyl Glutaramide) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

4-Arm PEG-MAL (Maleimide) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

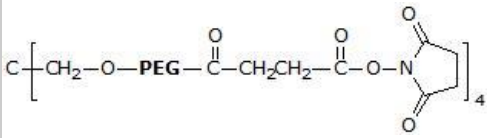
4-Arm PEG-ACLT (Acrylate) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

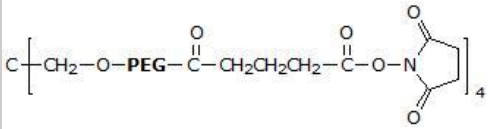
4-Arm PEG-SH (Thiol) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

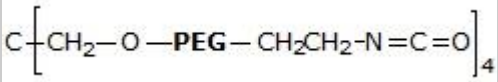
4-Arm PEG-VS (Vinylsulfone) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

4-Arm PEG-SS (Succinimidyl Succinate)	2K	Inquire
	5K	Inquire

NT-PEGYLu

	10K	Inquire
	20K	Inquire
	40K	Inquire

4-Arm PEG-SG (Succinimidyl Glutarate) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

4-Arm PEG-NCO (Isocyanate) 	2K	Inquire
	5K	Inquire
	10K	Inquire
	20K	Inquire
	40K	Inquire

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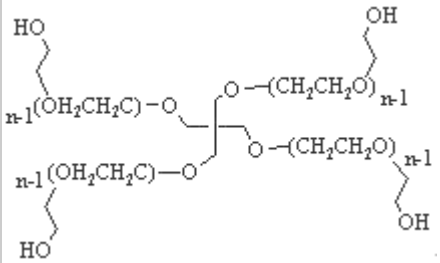
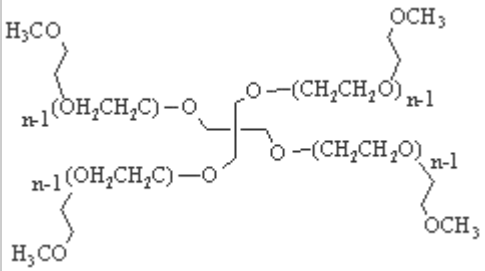
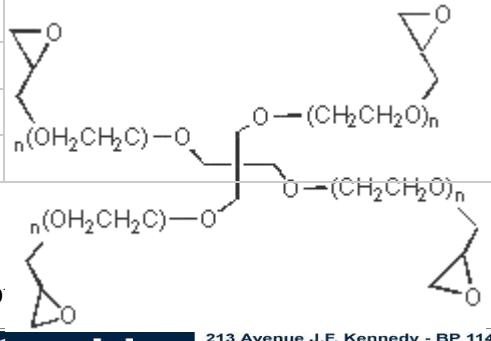
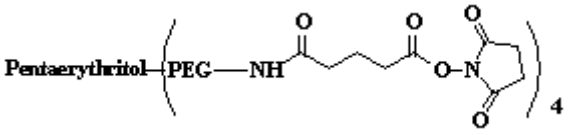
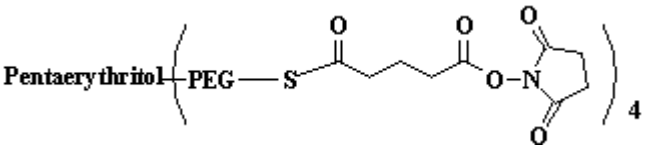
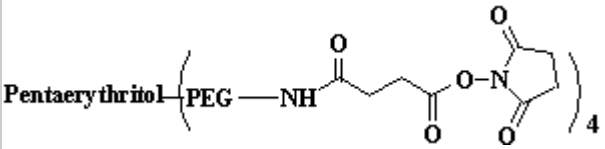
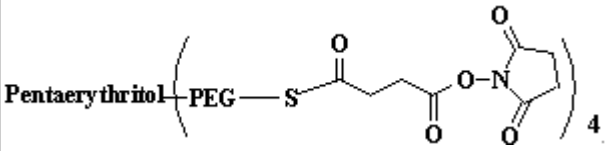
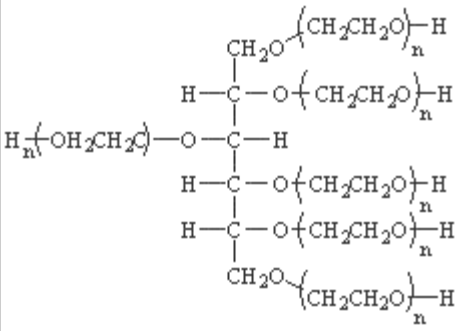
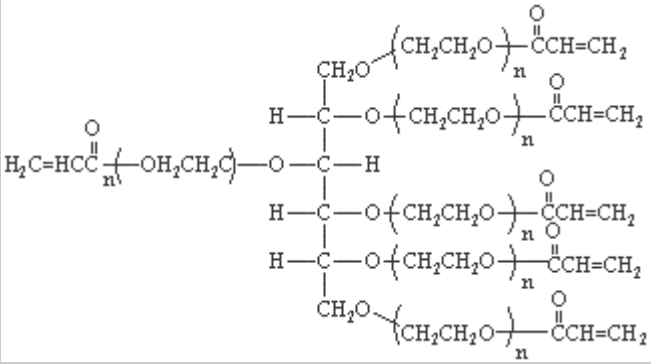
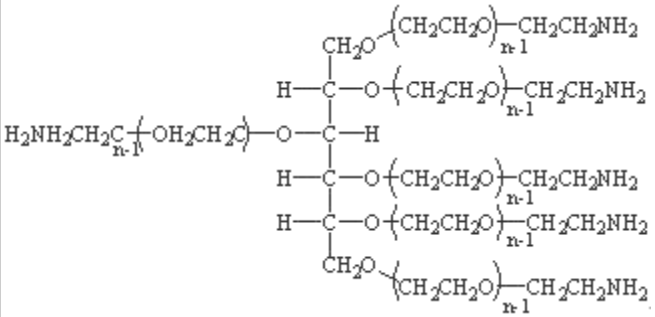
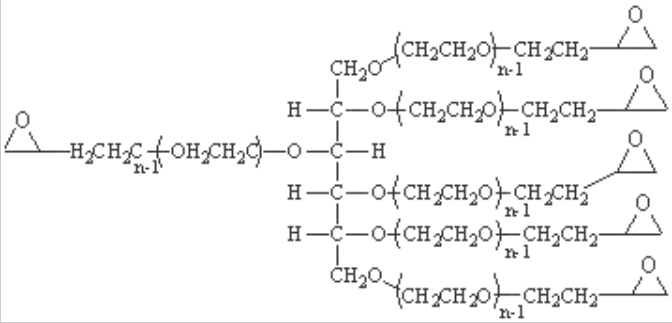
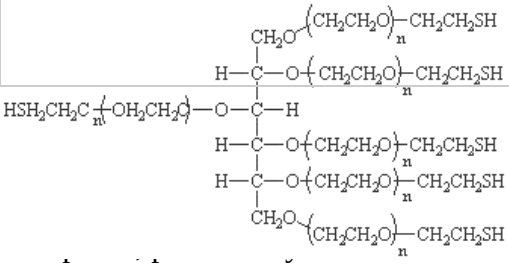
4-Arm PEG Derivatives		M.W.	Chemical Structure
4-Arm PEG-OH		10K	
		13K	
		15K	
		17K	
		20K	
4-Arm PEG-Methoxy		10K	
		13K	
		15K	
		17K	
		20K	
4-Arm PEG-Epoxide		10K	
		13K	
		15K	
		17K	

Contact your local distributor Up

		20K	
4-Arm PEG-amide-succinimidyl Glutarate		10K	
		13K	
		15K	
		17K	
		20K	
4-Arm PEG-thio-Succinimidyl Glutarate		10K	
		13K	
		15K	
		17K	
		20K	
4-Arm PEG-amide-Succinimidyl Succinate		10K	
		13K	
		15K	
		17K	
		20K	
4-Arm PEG-thio-Succinimidyl Succinate		10K	
		13K	
		15K	
		17K	
		20K	

Unique 6-arms PEG reagents

<*>

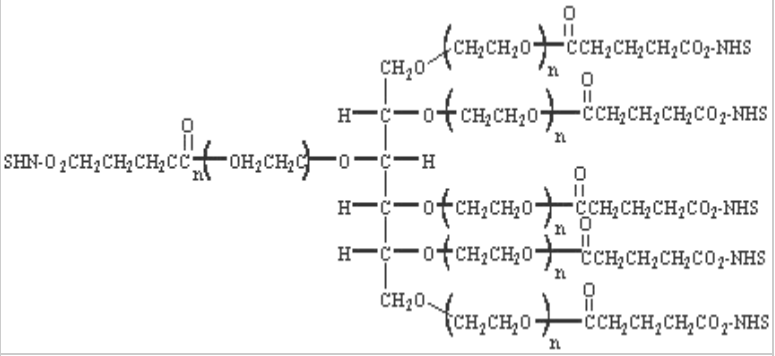
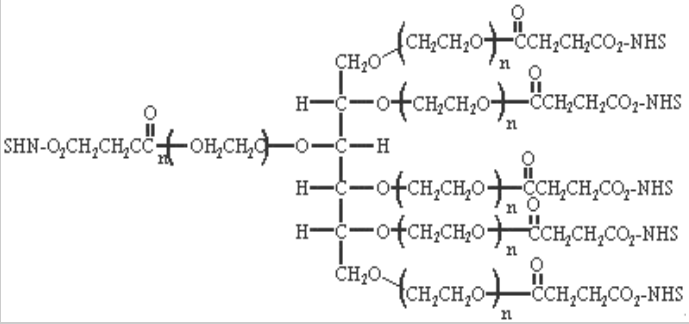
Product Name		M.W.	Chemical Structure
6-arm PEG-OH (raw material)		10K	
		15K	
		20K	
6-arm PEG-acrylate		10K	
		15K	
		20K	
6-arm PEG-amine		10K	
		15K	
		20K	
6-arm PEG-°epoxide		10K	
		15K	
		20K	
6-arm PEG-°sulfhydryl		10K	
		15K	

Contact your local distributor

uptima@interchim.com

interchim 213 Avenue J.F. Kennedy - BP 1140 03103 Montluçon Cedex - France Tél. 04 70 03 88 55 - Fax 04 70 03 82 60

NT-PEGYLu

		20K	
6-arm PEG-succinimidyl-glutarate		10K	
		15K	
		20K	
6-arm PEG-°succinimidyl °succinate		10K	
		15K	
		20K	
Other °6-arm PEG °derivatives		10K	Inquire
		15K	
		20K	

8-arm PEGs Derivatives

| back to other branched PEGs

8ARM-PEG-NH2: 8arm PEG Amine

8-Arm PEG-Amine (NH2) (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Carboxyl (hexaglycerol core)	
	10K
	20K
	40K

8ARM-PEG-COOH: 8arm PEG Carboxyl

8-Arm PEG-Carboxyl (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Carboxyl (hexaglycerol core)	
	10K
	20K
	40K

8ARM-PEG-MAL: 8arm PEG Maleimide

NT-PEGYLu

8-Arm PEG-Maleimide (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Maleimide (hexaglycerol core)	
	10K
	20K
	40K

8ARM-PEG-ACLT: 8arm PEG Acrylate

8-Arm PEG-Acrylate (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Acrylate (hexaglycerol core)	
	10K
	20K
	40K

8ARM-PEG-SH: 8arm PEG Thiol

8-Arm PEG-Thiol (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Thiol (hexaglycerol core)	
	10K
	20K
	40K

8ARM-PEG-SS: 8arm PEG Succinimidyl Succinate

8-Arm PEG-Succinimidyl Succinate (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Succinimidyl Succinate (hexaglycerol core)	
	10K
	20K
	40K

8ARM-PEG-SG: 8arm PEG Succinimidyl Glutarate

8-Arm PEG-Succinimidyl Glutarate (pentaerythritol core)	
	10K
	20K
	40K
8-Arm PEG-Succinimidyl Glutarate (hexaglycerol core)	
	10K
	20K
	40K

Dendrimers and dendrons

Dendrimers are composed of multiple perfectly-branched monomers emanating radially from a central core. The number of branches pincreases upon moving from the dendrimer core to its surface and defines dendrimer generation. The branched topology confers dendrimers with several unique properties for materials applications:

• **Compact structures**, from nm to µm scale

• **High solubility and low solution viscosity**. This property lends dendrimers to applications as rheology (viscosity) modifiers.

• **Multivalency**: multiple terminal groups on dendrimer surface can be displayed, even with high density increased generations ot dendrimers. Hence dendrimers allow concentrated payloads of drugs or imaging labels chemically grafted to the dendrimer surface.

• **Core-shell molecular architecture** can be used to encapsulate functional groups or bioactive compounds that chemically distinct from the surface, hence protected from the environment where they could be not stable or incompatible in the application. Notably,

-encapsulated drugs can be release in the environment of the dendrimer with time, upon particular environment change, or dendrimer clivage: for example catalysts, drugs, or chromophores.

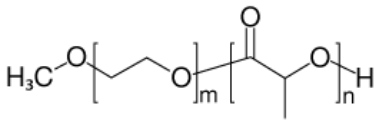
-internally grafter functional groups can be available to be active only after cleavage of the dendrimeric structure.

PEG-Like reagents (PLA, PCL,…)

Several structures homologous to PEG exist. They

Inquire for PLA-PEGs, PCL-PEGs

PLA-PEGs: Methoxy Poly(ethylene glycol)-block-poly-L-lactide



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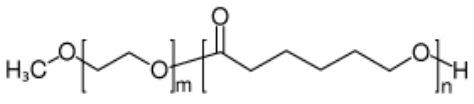
PCL-PEGs: Methoxy Poly(ethylene glycol)-block-poly(Ɛ-caprolactone)



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PVA: inquire [0].

Other polymeric spacers/backbones: biodegradable biopolymers

Many polymers have been developed alternatively to PEG, more or less close by their chemical structure and nature. Beside above PLA & PCL, aa-based polymers are the most popular for

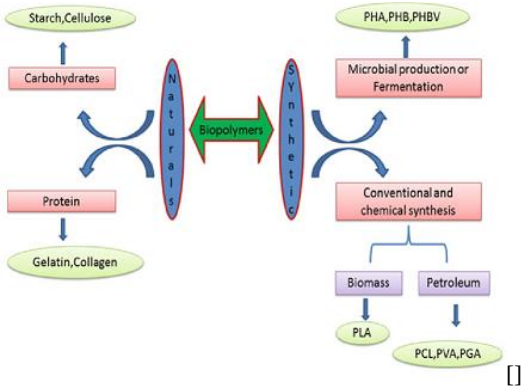
biomedical applications (e.g. as specialty backbone for drug delivery). Glycosidic polymers (e.g. cellulose-based) are more often useful for other industries, and several other natural derivatives or bioorganimimetics polymers exist. Such biopolymers achieve properties homologous or complementary to PEGs, and can be combined by conjugation, or by mixtures (gels, layers, …).

Tech tip – **Biopolymers**

Biopolymers are substances built from natural constituents or synthetic ones but with final biocompatible or bio-related properties. This is a strong trend known as white chemistry.

PEGs and related PLA, PCL belong the chemically synthetized, derived from fossil fuels or biomass.

PGA and other aa-based are also chemically synthetized, by par peptide synthesis using natural aa, and they key benefit is their biological nature making them even more biocompatible and biodegradable.



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>Key applications include

•Drug Delivery System: polymer micelles, polymersomes, chemical modification of drugs, etc.,

•biodegradable medical device applications: cell adhesion, and surface coating, hydrogels, etc.

•Packaging

>By natural sources:

-**Starch** derived and composites bio(nano)materials

-**Chitosan** derived and composites bio(nano)materials

-**Polylactic acid** derived and composites bio(nano)materials

-**Cellulose** derived and composites bio(nano)materials (incl. Carboxymethyl cellulose (CMC), Acetate ester C., …)

-dérivés de **tannins** : ()

-**Cotton** derived and composites bio(nano)materials

>Properties (desirable, or not)

-Degradability

- Gas and Moisture Obstruction Properties

- Thermal behavior / stability. Investigated by Thermogravimetric Analysis (TGA).

- Mechanical Properties: tensile properties, hardness, toughness, ductility. Investigated by usually destructive methods.

aa-based biopolymers: PGA, POR, PArg, PolySarcosine, PAS

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Crosslinker for gelification: bis-Acrylamide, PDA, DATD

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Crosslinker for Label Transfert: BASED, SBED,…

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Hetero Bi-Functional PEG Derivatives

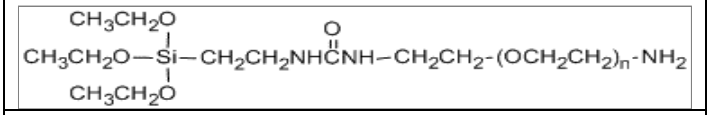
Functionalized PEGs for crosslinking (simple reactive groups, reactive groups)

FT-1A6350 (Hydroxyl-PEGx-NHS)
FT-WU0642 (NHS-PEGx-Thiol)
FT-WU0730 (Amine-PEGx-Maleimide)
FT-WU0760 (Carboxyl-PEGx-Maleimide)
FT-WU0651(Amino-PEGx-Hydroxyl) FT-BW7631 (Amine-PEO-Hydroxyl)
FT-B48ZD1 (Amino-PEGx-Carboxyl) FT-AXGYOA (Amino-PEG-Carboxyl) FT-AN1281
*Carboxyl + x
FT-B48ZD1 (Amino-PEGx-Carboxyl) FT-AXGYOA (Amino-PEG-Carboxyl)
FT-GO5231 (Carboxyl-PEG-Hydroxyl)
FT-WT9771 (Carboxyl-PEGx-Thiol)
*Thiol + x
FT-WT9761 (Amine-PEGx-Thiol)
FT-1I6661 (Azide-PEGx-Thiol) FT-AXGZ11(Azide-PEGx-Thiol)
FT-WT9771 (Carboxyl-PEGx-Thiol)
FT-T9781 (Hydroxyl-PEGx-Thiol)
FT-WU0642 (NHS-PEGx-Thiol)
*Hydroxyl + x
FT-GO5231 (Carboxyl-PEG-Hydroxyl)
FT-1A6350 (Hydroxyl-PEGx-NHS)
FT-T9781(Hydroxy-PEG-Thiol)
*Hydrazide + x
FT-B0CDF1 (Hydrazido-PEGx-XXX)
*Azide + x
FT-WU0910 (Amine-PEG-Azide) FT-C5015T: Azide-PEO$_x$-Amine
FT-ANGIX0(Azido-PEG12-Acid)
FT-1I6661 (Azide-PEGx-Thiol) FT-AXGZ11(Azide-PEGx-Thiol)
FT-WU0930 (Azide-PEG-Carboxyl)
*Clickables groups + x
FT-AWJKP0 (Alkyne-PEGx-Hydrazide)
FT-DQP580 (DBCO reagents)
FT-MRU990 (TCO-PEG4-NHS ester)
*Protected groups and others + x
FT-RPW480 (Boc-NH-PEG-COOH)
FT-AXEST1(mPEG-X)

Silane PEGs

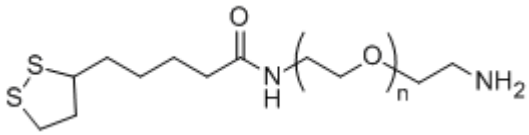
Cat.Number	Name

NT-PEGYLu

(See FT-LO5310)	SILANE – PEGx – CARBOXYL  SILANE – PEGx – AMINE SILANE – PEGx – AMINOOXY SILANE– PEGx – CARBOXYL SILANE– PEGx – NHS (an SS, GS…) SILANE– PEGx – MALEIMIDE SILANE– PEGx – AZIDE SILANE– PEGx – HYDROXYL SILANE– PEGx – THIOL SILANE– PEGx – FOLATE SILANE– PEGx – BIOTIN SILANE– PEGx – FITC

Lipid PEGs: DSPE , LipoicAcid,…

FT-0A5061 (LipoicAcid-PEGs)

Cat.Number	Name
(See FT-0A5061)	LIPOIC ACID – PEGx – AMINE (LA-PEG-NH_2)  LIPOIC ACID – PEGx – AMINOOXY LIPOIC ACID – PEGx – CARBOXYL LIPOIC ACID – PEGx – NHS (an SS, GS…) LIPOIC ACID – PEGx – MALEIMIDE LIPOIC ACID – PEGx – AZIDE LIPOIC ACID – PEGx – HYDROXYL LIPOIC ACID – PEGx – THIOL LIPOIC ACID – PEGx – FOLATE LIPOIC ACID – PEGx – BIOTIN LIPOIC ACID – PEGx – FITC

FT-LD454A (DSPE-PEGx-Folate)
FT-1I7901 (DSPE-PEG_Folate)
FT-JO2570 (DSPE-PEG-Mal)
FT-1B2940 (Folic Acid-PEG-Hydroxyl)
FT-1B2980 (Folic Acid-PEG-Amine)

Biotin PEGs

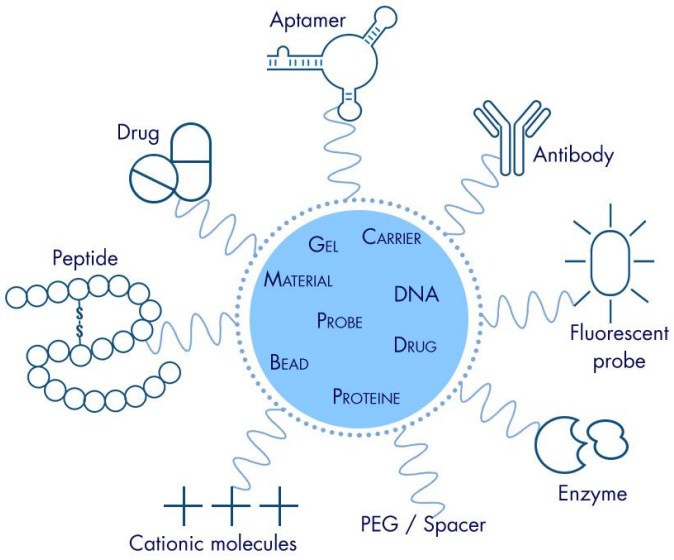
FT-DZ5520 (Thiol-PEG-Biotin)
FT-IU3901 (Azide-PEG-Biotins)
FT-B36H81 (Biotin-PEGx-Azide)
FT-R2028A (Maleimido-PEOx-Biotins)
FT-78631A (Hydrazide-Biotin)
FT-FJ675.1(Biotin-Azide)

Fluorescent PEGs

FT-1A7400 (Rhodamine-PEG-thiol)
FT-1B7350 (CYanine5-PEG-Thiol)
FT-1B7360 (FITC-PEG-Thiol)

Multivalent PEGs (Branched, Multi-Arms, Dendrimers)

*Applications of PEGylation



PEO reagents that are activated with specific functional groups (reactive groups, bulky groups, labels, ligands...) have found diverse applications, including organic synthesis (Building blocks, Linkers, i.e. for peptide or synthesis), surface modification, microarray, probe preparation (crosslinkers), labeling (Biotin, Fluorescent labels), vaccines, biomedical materials,...

Beside chemistry works (organic synthesis, biochemistry), PEG compounds find great applications in industry, starting cosmetics but also improved and new materials, medicines, Pharma industry…

Chemical Synthesis (peptides, oligonucleotides)

Some PEG compounds can be used as building block in the synthesis process. This include blocked functional groups :

Search PEG-Fmoc[0], -tBoc[0], Cbz[0],…
See other Protecting groups in (bio)chemistry[].

Biochemistry

PEG compounds are typically used for biochemical modification of any molecules, starting with biomolecules. See above sections.

• Pegylation of biomolecules by target functional groups:
amines, carboxyls, hydroxyls, thiols, Clickable groups,… N-terminal amino acids of peptides

• Pegylation of surfaces :
Gold, Glass and silicones, other tips

• Special pegylation approaches:

Click-type methods, Branched pegylation, Reticulation,

Cosmetics

PEGs are used in cosmetics as the basic compounds or in combination with their derivatives (“as is”), mono-, di- and poly-esters, amines, ethers and acetals but also others.

Since many PEG types are hydrophilic, they are favorably used as penetration enhancers, especially in topical dermatological preparations. Polyethylene glycols (PEGs) and their derivatives are widely used in cosmetics as surfactants, cleansing agents, emulsifiers, skin conditioners, and humectants.

Most PEGs are polyethylene glycol ether propylene glycol, i.e. PEG-10 Propylene Glycol. But many other derivates are available:

-polyethylene glycol (PEG) **ethers of propylene glycol stearate**: i.e. PEG-25 to -120 Propylene Glycol Stearate

-polyethylene glycol **ethers of propylene glycol cocoate** (fatty acids derived from coconut oil): i.e. PEG-8 Propylene Glycol Cocoate

-polyethylene glycol **ethers of propylene glycol oleate**: i.e. PEG-55 Propylene Glycol Oleate

Improved and new materials

PEGs (ant their derivatives) are widely use as surface modification agent to improve the properties of both natural and synthetic materials. Modified properties include hydrophilicity, electric constant, softness/stiffness, UV resistance,… Quite any material are have been pegylated, from hard material like gold, glass and metals, to more flexible or porous ones like thermoplastics, resin or cellulose.

Medecine & Pharma industry

PEGs are already employed for the manufacturing of numerous medical devices (as surface modification agent) and even therapeutic agents (ADC, see below) or diagnostics (as probe linkers, as surface modification agent).

Linker/modifier for Drug Conjugates (incl.ADCs) []

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See Lipidic PEGs.

See Clickable PEGs.

Linker/modifier for Drug Conjugates (incl.ADCs) []

Use of PEG-DSPE block copolymers as nanomaterials in drug deliver

Antibody Drug Conjugates (ADCs) comprise typically an antibody (mAbs) specific for a target (biomarker of a -tumorous- cell line), an active cytotoxic drug (toxine), and an appropriate linker.

-the drug is surely the first intended active component, typically a toxic payload.
-the antibody is used to target and deliver a toxic payload to the selected diseased tissue. The Ab can also in nsome instance be the bioactive drug!
-the linker confers to the conjugate (of drug, ab, particle) hydrophilicity and flexibility or rigidity between linked components. It should avoids steric hindrance.

NT-PEGYLu
ADC are finally designed to improve drug activity and distribution in body and organs, decrease drug clearance or degradation, or release drug.
when not including an antibody, a conjugate is called **PDC** for Protein or Peptide-based conjugates.

Variants of ADCs, can
.replace the antibody by other probes (lectin,…)
.replace the drug by a contrasting agent, a radioactive element, nanoparticles, an enzyme or receptor substrate or inhibitor,…
They all are designed to improve the potency and effectiveness of the bioactive and accessory partner, drug or cytotoxic agent, antibody, particles.

Antibody Drug Conjugates are a rapidly expanding area in the pharmaceutical industry. The majority of the **ADCs** currently under development or in clinical trials are for oncological and hematological indications. There are over 30 ADCs currently in pre-clinical or clinical development. This is primarily driven by the availability of monoclonal antibodies targeting various types of cancer. However, some drug developers are also looking to expanding the application of ADCs beyond oncology and hematology to other important disease areas.

Compared to heterogeneous ADCs, the analogous homogeneous ADCs have been prepared by **site-specific linkage to antibodies** because they exhibit reduced toxicity and superior efficacy in vivo. Such homogeneous ADCs have been obtained via the technology of interchain cysteine cross-linking. In recent years, some novel cysteine-reactive functionalities have been developed to introduce specially designed functional groups at the native disulfide bonds antibody fragments or full antibodies, such as pyridazinedione, dibromopyridazinedione, dibromomaleimide, and bis-alkylating bis-sulfone groups. Other recombinant approaches can introduced particular tags or aminoacid giving site-located conjugations.

Genetic engineering of the mAb allows to introduce cysteines or non-natural amino acids with an orthogonally-reactive functional group handle such as an aldehyde, ketone, azido, or alkynyl tag. These site-specific approaches not only increase the homogeneity of ADCs but also enable novel bio-orthogonal chemistries that utilize reactive moieties other than thiol or amine.

The cytotoxic drug, monomethyl auristatin E (MMAE), is conjugated to the three trastuzumab variants using a protease **cleavable linker** to release drug in target organ, and shows in vivo therapeutic efficacy. The linker-MMAE conjugate is used in the U.S. FDA approved ADC, Brentuximab vedotin. There are also ADCs adopting linker-MMAE conjugate under clinical trials, such as Enfortumab vedotin and Glembatumumab vedotin.

Poly(ethylene glycol)–distearoylphosphatidylethanolamine (PEG-DSPE) block copolymers are biocompatible and amphiphilic polymers that can be widely utilized in the preparation of liposomes, polymeric nanoparticles, polymer hybrid nanoparticles, solid lipid nanoparticles, lipid–polymer hybrid nanoparticles, and microemulsions. Particularly, the terminal groups of PEG can be activated and linked to various targeting ligands, which can prolong the circulation time, improve the drug bioavailability, reduce undesirable side effects, and especially target specific cells, tissues, and even the intracellular localization in organelles.

Carboxyl groups are introduced to the terminal groups of PEG-DSPE block copolymers, which can easily react with the ligands for active target cells or tissues, such as transferring and peptide.[]

See Lipidic PEGs.

Most PEG are manufactured for large polymer as polysidpserse compounds. The moleculae dispersity is measured by a "dispersity index" (Đ), formerly known as the polymer dispersion index (PDI), typicall 3-10%. Lower molecular weight polymers have a larger Đ than those of higher molecular weights. However, no matter the dispersity index, the molecular weight species have a Poisson distribution that forms a bell-shaped curve. It has been shown that modest differences in the number and configuration of ethylene glycol units can modulate conjugate properties and *in vivo* pharmacokinetic performance.

Synthetic single-molecule PEGs, designated as PEO (for PolyEthylenOxy), are perfectly defined unique structures. Their use increase the quality of formed conjugates, and simplifies greatly their characterization (by LC, MS, RMN,…), that is an important step in the development process of ADCs. In particular HighResolution MS allows to confirm the monoisotopic mass not only of PEOs and their small MW conjugates according the expected chemical formula, but large conjugates such as a PEO-Antibody: ESI/LC/MS can identify conjugates, after deconvulation, with 4 to 10 coupled PEO_{12} adducts! while the Ab conjugate made with a PEG1KD has a complexe MS profil which show a cluster attributed to 5-7 coupled ratio conjugates, with remaining large deconvoluted peaks that make unlikely that these peaks represent real conjugates.

Hence, limiting heterogeneity of the raw materials using single-molecule PEG compounds (PEOs), along with using established and appropriate analytical methodologies early in the development process, greatly facilitates the the characterization to suit the most critical considerations for gaining regulatory approval of INDs.

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Delivering molecular therapeutics efficiently and specifically to the targeted tissue remains a significant challenge.

See the TechnicalNotice about ADC/PDC techniques and applications.

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More information

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

Rev.A11E-N01E