The dPEG® Linker Platform for BioDesign™ of Bioconjugate Therapeutics

Introduction

The term dPEG® stands for "discrete PEG", which is a uniform, single molecular weight (MW), highly pure, next generation polyelthylene glycol polymer. Vector Laboratories manufactures dPEGs using proprietary, patent-protected processes to provide specific MWs, reactive groups, functional moieties, and architectures suited for a wide variety of applications (see Figs 1 & 2). It has been well-established that PEG itself is inert, non-toxic, water soluble, and biocompatible, and when these properties are combined with the above-mentioned features of dPEGs, it provides a powerful tool for the design, optimization, and development of bioconjugate therapeutics.

Explore the dPEG offerings from Vector Laboratories

Array of dPEG based products for research and development —

Numerous synthetic technologies, chemistry expertise and deep familiarity with customer needs have enabled Vector Laboratories to develop a broad portfolio of PEG product lines that are diverse, highly pure, and scalable.

- Homo-, hetero-, and multifunctional crosslinking reagents for conjugating biologics, payloads, carriers, and surfaces.
- A wide variety of reactive groups for conjugation strategies including click chemistry, biorthogonal, site-specific, enzymatic, and stochastic approaches.
- Building blocks & intermediates for flexible linker architecture design.
- Chemical modification reagents with a variety sizes, architectures, and end capping.
- Block copolymers for polymeric and lipid nanoparticles.
- Affinity tags including biotin, lipids, and haptens.
- Fluorophores with increased hydrophilicity.

Figure 1

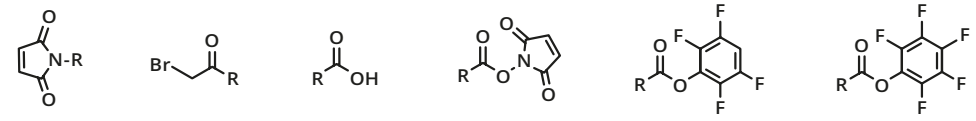


Figure 1

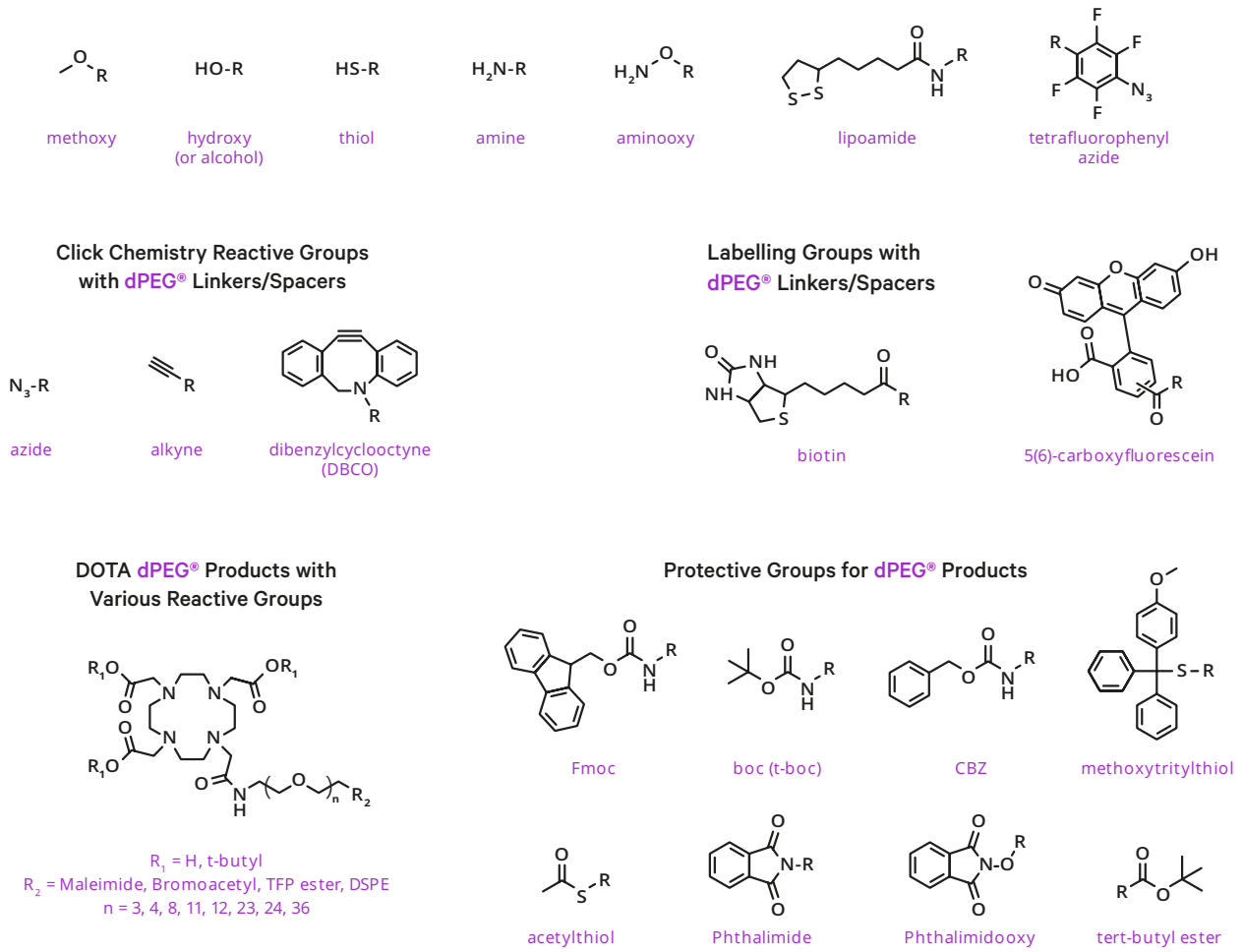


***Figure 1**: Examples of the functional, reactive, labeling, and protective groups on dPEG® products.*

Figure 2

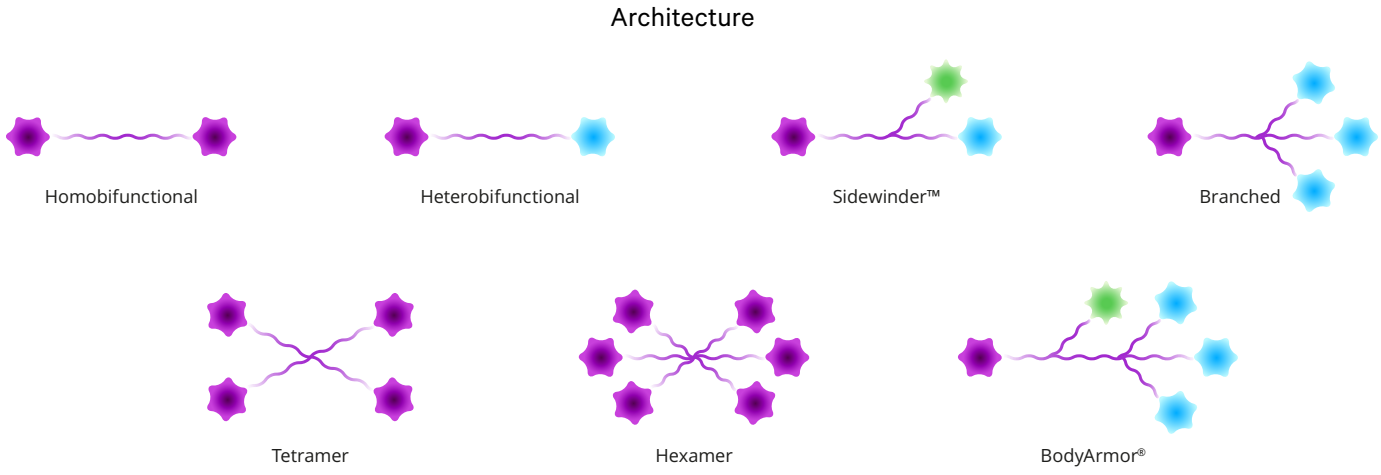


***Figure 2**: Available architectures for dPEG® products. Branched dPEG® products can have three (3) or nine (9) branches. Our Sidewinder™ products are a new class of dPEG® constructs that offer a broad range of new ways to incorporate dPEG® functionality into diagnostic and therapeutic applications. The BodyArmor® product architecture is similar to the Sidewinder, but includes additional orthogonal dPEG® strands.*

Comparison of traditional PEGs with dPEGs

The first clinical applications of large, polydisperse, traditional PEGs in drug development were the PEGylation of proteins, peptides, and enzymes (Oncaspar, Adagen, Peg-Intron, etc.) to improve their drug metabolism and pharmacokinetic (DMPK) properties. This strategy of altering the physiochemical (PC) or DMPK properties by the covalent attachment of PEG is now used for several therapeutic modalities including antibody fragments, peptides, small molecules, oligonucleotides, and nanoparticles.

The dPEGs or uniform PEGs from Vector Laboratories can be used to further optimize the PC and adsorption, distribution, metabolism, elimination, and toxicity (ADMET) properties of a therapeutic, to achieve targeting, solubility and stability requirements. Table 1 shows a few advantages of uniform PEGs over traditional PEGs.

Traditional Polydisperse PEGs	dPEGs
Lower purity with multiple MW entities	Highly pure with a single MW entity (see Fig. 3)
Manufactured by a polymerization process	Manufactured by a proprietary process
Complex to analyze due to multiple entities	Straightforward analysis
Limited design opportunities due to multiple entities	Infinite design possibilities for bioconjugation tools
Limited flexibility of BD, PD and PK property modulation for any bioconjugate constructs	High flexibility for optimization of BD, PD and PK properties in all bioconjugate constructs due to design and manufacture controls

***Table 1**: General differences between traditional PEGs and dPEGs.*

Figure 3

Side-by-side mass spectra of traditional polyethylene glycol (PEG) and a dPEG® of equivalent mass from Vector Laboratories

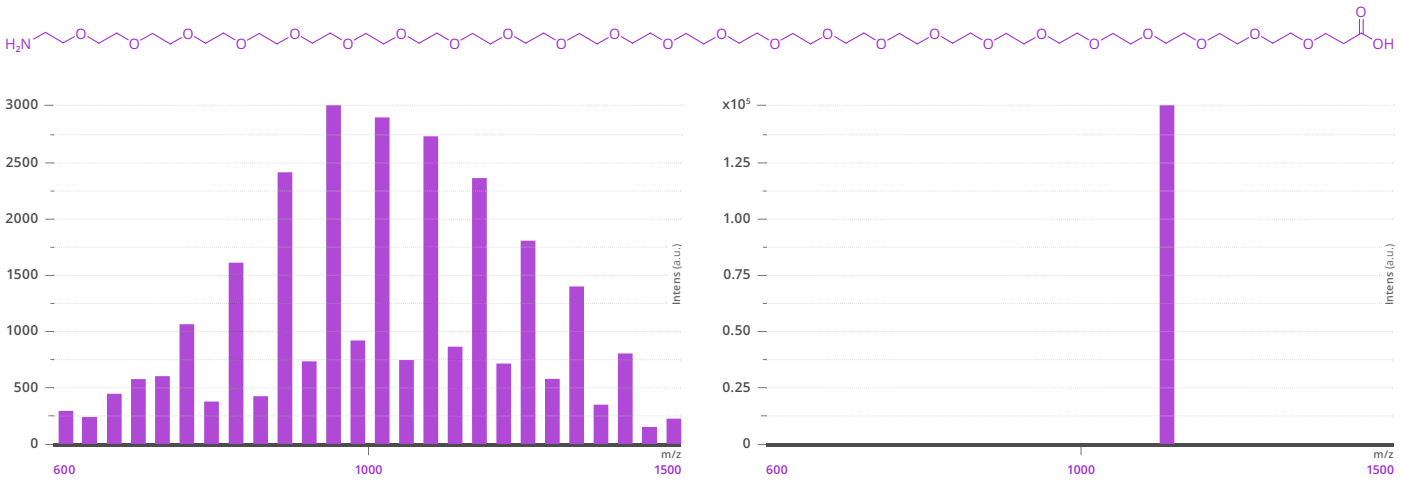


***Figure 3**: Side-by-side comparison of actual mass spectra from a traditional, dispersed PEG (left spectrum) and a dPEG® of equivalent mass from Vector Laboratories (right spectrum). The mass spectrum on the left is of PEG1000. It has M_w = 1027 Daltons; M_n = 888 Daltons; and Đ = 1.16. The masses in this dispersed PEG range from 600 – 1,500 Daltons. The mass spectrum on the right is of Vector Laboratories product number QBD-10317, amino-dPEG®$_{24}$-acid, the structure of which is shown across the top of the two mass spectra. QBD-10317 is a single molecular weight compound with a single, discrete chain length. The molecular weight of QBD-10317 is 1146.355 Daltons. Because it has no dispersity, Đ = 1.*

Wide Variety of Applications with dPEGs —

Due to the unique combination of biocompatibility, uniformity, and designability, dPEGs can be used to impart favorable properties in a wide variety of applications including:

- Linkers for conjugates such as antibody drug conjugates (ADCs), fragment drug conjugates (FDCs), protein drug conjugates (PDCs), small molecule drug conjugates (SMDCs), oligo conjugates (OCs), and drug delivery systems (DDS), without the hydrophobic liabilities of alkyl linkers and ambiguity of polydisperse linkers ***(see Table 2)***.
- Spacers and spatial modifiers, to explore and optimize proximity effects with a high degree of flexibility.
- Surface modifiers to alter size, shape, charge, hydrophobicity, permeability, and impart stimuli or temporal dependent qualities in small molecules, oligonucleotides, peptides, proteins, antibodies, polymers, dendrimers, lipid nanoparticles, and inorganic surfaces/ nanoparticles.

Property	Polydisperse PEG	Alkyl cross linker	Sulphonated cross Linker	Uniform PEG
Purity	+	++++	++++	++++
Manufacture process	Polymerization	Synthetic	Synthetic	Proprietary
Analysis	Complex due to multiple entities	Simple	Simple	Simple
Flexibility of Design	+	+	+	++++
Flexibility of modulation of PK, PD properties in bioconjugate constructs	+	+	+	++++
Hydrophilicity	+	+	+	++++
Ability to shield hydrophobic moieties	+	+	+	++++

Table 2*: Comparison of Crosslinker Types.*

The following section shows a few advantages of using uniform PEGs or dPEGs over traditional PEGs and alkyl crosslinkers in various moieties.

dPEGs as Linkers

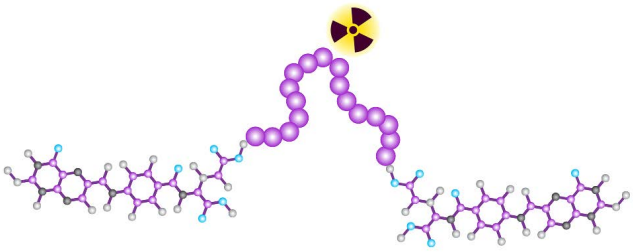
The ability to conjugate different entities together with crosslinkers has proven to be an immensely useful technology for diagnostics and drug delivery systems. Crosslinkers comprised of polydisperse PEG have been used to make multimers and to conjugate targeting ligands to nanoparticles. This is generally required for applications where a very large size is required to provide favorable DMPK properties and is less affected by polydispersity. However, some properties, like target binding or cellular internalization, can be adversely affected by the large size. There is also the potential for therapeutic sensitivity to changes in the PEG chain length. Therefore, smaller uniform dPEGs can provide effective solutions
in such cases.

Traditional crosslinkers comprised of small alkyl groups (such as SPDP, SIAB, SMCC, EMCS, etc) have also been the mainstay of bioconjugation for many years, however their inherent hydrophobicity creates significant limitations as the conjugate design becomes more sophisticated. For e.g., when multiple entities are conjugated together or proximity analysis is performed, the linker component can be critical to overcome inherent hydrophobicity with some hydrophilic modifications. Several alkyl linkers are available in sulfonated forms, and while this can improve the water solubility of smaller linkers, their application to larger constructs is questionable. In addition, the presence of many negatively charged sulfonates can contribute to non-specific interactions.

The dPEG product portfolio combines the precision of alkyl linkers with the biocompatible properties of PEG, without any hydrophobic liabilities, with a high degree of flexibility, and with a variety of unique architectures to determine structure-function relationships and optimize conjugate properties. In the below examples, we show some advantages of uniform / dPEG cross linkers.

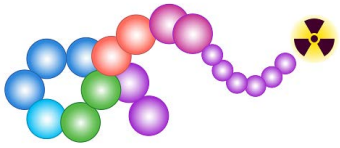
Small molecule conjugates

There are several studies that document the advantages of using dPEGs to link small molecules to imaging agents for diagnostic applications. For instance, when folate dimers labelled with fluorescein were prepared with 2 kDa PEG, 1 kDa PEG, or dPEG24 crosslinkers, the dimers with the dPEG24 crosslinkers exhibited the highest cellular uptake. In another study on peptide multimers, two dimers of PCMA



targeting ligands were conjugated to DOTA via two uniform PEG4 linkers to provide a tetramer that had improved PK and tumor targeting than the dimeric and monomeric conjugates.

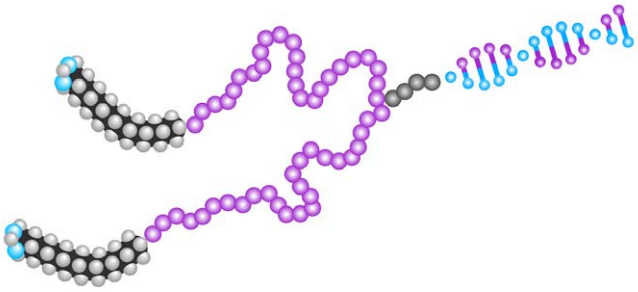
Peptide Conjugates



Research has shown that dPEG spacers can improve the properties of peptide drug conjugates. For instance, it was noted that the solubility of an RGD-cryptophycin conjugate could be improved by the inclusion of a uniform PEG4 spacer, and the payload release from an RGD-Glu-MMAE conjugate could be facilitated by the inclusion of a uniform PEG4 spacer.

Oligo Conjugates

Delivery has become the major hurdle for oligos, and as such several conjugates have been designed as delivery systems that incorporate dPEGs as cross linkers including antibody conjugates, peptide

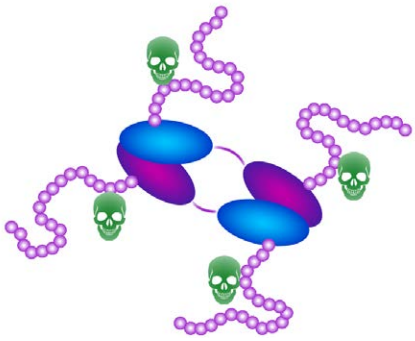


conjugates, and lipid conjugates. For instance, there are a variety of lipid-ON conjugates the incorporate both linear and SideWinder® type uniform PEG4 spacers to modulate the PK behavior and trans-membrane delivery.

Degraders and Degrader Conjugates

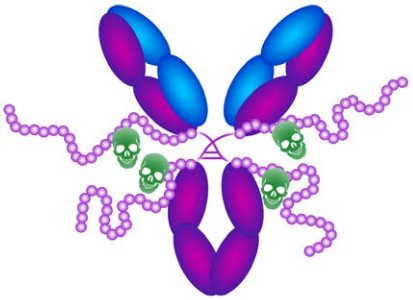
Proximity-induced degradation is a promising new modality for drugging the undruggable targets, and PROTACs are one of the more popular classes of degraders. The linker between the ligands for the ligase and the protein of interest are typically alkyl linkers or short uniform PEG4 linkers, and they play a critical role in PROTAC development. The length of the dPEG can be optimized for efficient degradation, and some dPEG-based linkers have facilitated the design of "clickable" PROTACs for more rapid optimization of ligand combinations.

Fragment Conjugates



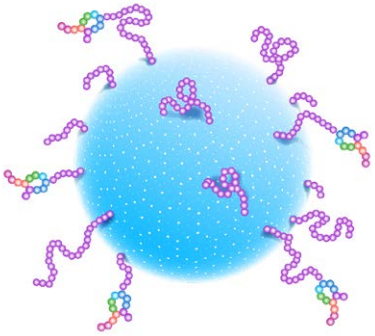
Fragment conjugates are being developed with the promise of overcoming issues related to the large size of antibodies, such as poor extravasation and tumor penetration. Due to the smaller size of antibody fragments, the linker's properties can play a larger role in modifying the properties of the conjugate. For instance, a TAG72 targeting diabody was crosslinked to DOTA using dPEG spacers of different lengths. The dPEG spacers were able to increase the hydrodynamic volume of the diabody to reduce kidney clearance and improve tumor uptake, and a dPEG48 spacer provided the highest tumor/kidney ratios. Vector lab's SideWinder® crosslinkers have also proved beneficial for diabody-TCO-MMAE conjugates where the smaller size of the biologic required an orthogonal PEG modifier to prevent in vivo deactivation of the TCO trigger.

Antibody Conjugates



ADCs are leveraging the properties of dPEG spacers to offset the liabilities of hydrophobic payloads and improve PC, PK, BD, and toxicity. While linear dPEG crosslinkers have provided payloads with reduced hydrophobicity (e.g., tesirine), Sidewinder like crosslinkers have been shown to increase tumor uptake, reduce off-target uptake, and improve tolerability of ADCs with a variety of payloads. In addition, BodyArmor™ like linkers have been shown to provide additional structural variables that can be used to control payload protection and enzyme-mediated payload release.

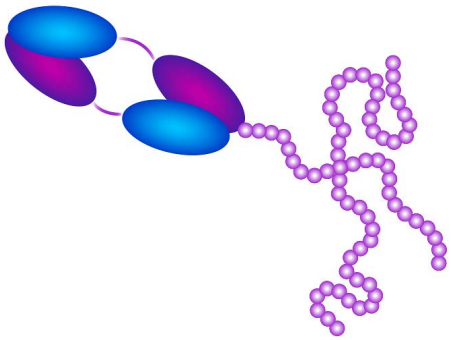
NP Conjugates



NPs typically improve drug delivery via passive targeting, but active targeting is being explored through the conjugation of targeting entities. Traditionally this has been accomplished with polydisperse PEG linkers, however both antigen binding and internalization are affected by the number of EO units in the PEG coating and the crosslinker. A study on targeted micelles and liposomes using dPEG crosslinkers of different lengths to conjugate peptides targeting either integrin or HER2 found that cellular internalization was dependent on the dPEG length, the targeted antigen, and the type of NP. The differences were noticeable for changes as small as 4 ethylene oxide units, and optimizing the performance of targeted NPs with this degree of granularity is only possible with dPEGs.

Covalent Attachment of dPEGs as Modifiers of PC and ADMET Properties

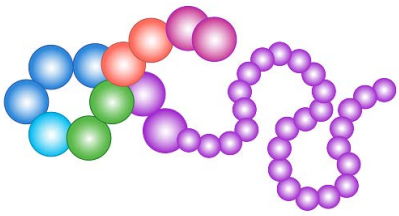
Antibody fragments



PEGylation of engineered antibody fragments has typically used larger polydisperse PEG (Cimzia, Dapirolizumab Pegol, etc) to prolong the half-lives of the small proteins. Recently, Genentech researchers noted that the conjugation of a variety of Fab conjugates to a 16 kDa branched dPEG (QBD-11487) resulted in a hydrodynamic radius of ~ 5 nm, which is above the generally accepted size cut-off for renal filtration. Researchers at Astra Zeneca prepared a set of diabody conjugates and noted that the size of a diabody can be increased from 2.9 nm to 3.3 nm by conjugation to a smaller branched 5 kDa dPEG (e.g., QBD-11471). While the conjugate still exhibited significant renal accumulation, the T½ increased by 7.5x relative to the parent Fab and it had a more favorable tissue/tumor ratio in lung, spleen, and skin relative to conjugates with larger polydisperse PEGs.

Peptides

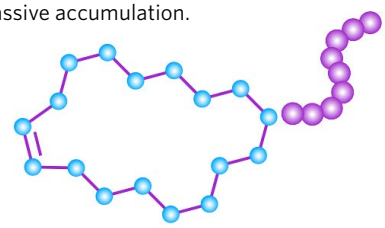
PEGylation or covalent attachment of PEGs to peptides, remains an attractive approach for improving DMPK properties of peptides. Some peptide-PEG conjugates currently in clinical trials include exenatide, adrenomedullin, and sihematide. Covalent attachment of smaller



uniform PEGs like dPEGs, to peptides to alter their physicochemical properties and ADMET profiles has seen more success recently. For instance, preclinical studies have shown that conjugation of dPEG24 (QBD-11304) to both galanin and NPY peptides decouples central and peripheral actions by preventing them from crossing the blood brain barrier (BBB), while facilitating analgesic activity. Incorporating a uniform PEG24 into the structure of Zilucoplan was also shown to impart favorable PC & DMPK properties, and this therapeutic recently received FDA approval for the treatment of generalized myasthenia gravis (gMG) in adult patients.

Small molecules

Early preclinical studies generally employed large PEG as a carrier to prolong the half-life of small molecules subject to renal clearance, improve the aqueous solubility of small hydrophobic molecules, and improve tumor targeting via passive accumulation.



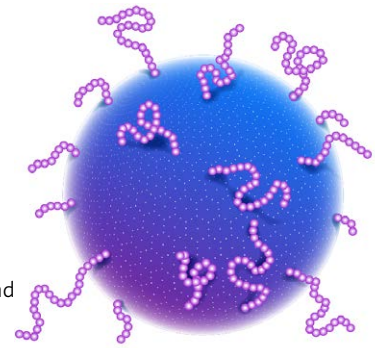
There are several successful cases where small dPEGs can be conjugated to small molecules to improve the PC properties or in vivo behavior. For instance, Movantik, approved in 2014, uses a small uniform PEG7 conjugated to the μ-opioid antagonist to prevent crossing of the BBB to facilitate treatment of opiod-induced constipation (OIC) without central nervous system activity.

Oligonucleotides (ONs)

While many of the potential liabilities of therapeutic ONs are addressed with chemical derivitization of the nucleosides or phosphate backbone, a variety of them, including antisense, aptamer, siRNA, and miRNA have been PEGylated. Studies found that a short dPEG12 could be conjugated to either the sense or the antisense strand of siRNA with no detrimental effects on mRNA knockdown, and a short dPEG12 conjugated to both phosphodiester and phosphorothioate antisense agents had no detrimental effect on gene silencing ability. Thus, small dPEGs may be able to enhance ON chemical derivatization.

Nanoparticles (NPs) and Drug Delivery Systems

Nanoparticles come in a variety of formats including dendrimers, polymeric nanoparticles, lipid nanoparticles, and inorganic particles. While they each have specific considerations, PEGylation of the nanoparticle surface has proved beneficial for most formats by preventing recognition and clearance by the immune system and reducing non-specific adsorption of serum proteins. There are several PEGylated NPs in clinical use (Spikevax, Comirnaty, etc) and many more in clinical trials (such as Promitil, ThermoDox, etc), all of which employ a 2 kDa PEG. While this has typically been the realm of polydisperse PEGs, dPEG products are available with this MW and numerous studies have shown that variables including PEG length can be beneficial the adsorption of serum proteins, transport through mucus and ECM, and the accelerated blood clearance phenomenon.



dPEG at the crossroads of Chemistry and Biology

In conclusion, the dPEG technology is at the foundation of numerous bioconjugate therapeutics and clinical diagnostic assays. Numerous publications have showcased the utility of dPEG based linkers and cited better target specificity, improved tumor uptake and lower toxicities with such linkers.

Overall, the competitiveness of using dPEG as linkers, modifiers, block co-polymers or functional tags lies in its discreteness, which is not available with traditional PEG.

Vector Laboratories has the deep technical expertise and manufacturing capabilities to help you design and develop unique dPEG based entities for integration within your conjugation strategy. With the **BioDesign** service, we can provide you with personalized, expert guided consultation, that can take your bioconjugate therapeutics to the next level.

Explore BioDesign™

Get in touch with us at
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to take the next step in your bioconjugate journey.

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