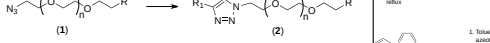
Synthesis and Facile End-Group Quantitation of Functionalized PEG Azides

J. Edward Semple, Bradford T. Sullivan and Kevin N. Sill, Intezyne Technologies, Tampa, FL 33612

POLY 267

Abstract

Functionalized PEG derivatives are finding ever-increasing applications in the areas of pharmaceuticals and targeted drug delivery by their judicious incorporation into nanoparticle-forming polymeric systems. We are investigating a range of novel mono- and bifunctional PEG azide derivatives that possess terminal hydroxyl-, carboxylate-, methoxy-, and (optionally protected)-amino moieties and sought to identify a simple, rapid, and robust method to quantitatively determine the level of azide substitution in our intermediates.



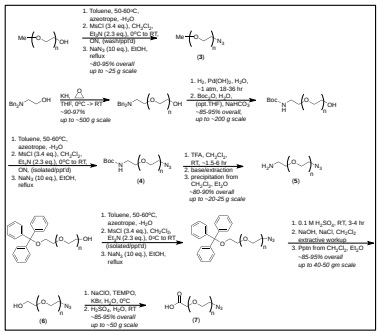
(**1**) (**2**)

In this poster, we describe the synthesis of a range of novel mono- and bifunctional azido-PEGs (**1**), their uncatalyzed click reactions with four dipolarophiles, and identification of optimal reaction conditions to afford triazoles (**2**), whose ^{1}H-NMR spectra may be conveniently utilized to quantitate their level of azide substitution.

Discussion

Click reactions have found widespread utility in both chemistry and biology applications.[1,2] The [3+2] cycloaddition reaction between azide and activated alkyne moieties generally proceeds under mild thermal or metal-catalyzed conditions and produces stable triazole adducts in high yield. Mono- and bifunctional PEGs featuring azide, alkyne, alcohol, and carboxylate moieties are particularly useful for bio-orthogonal click reactions (conjugations, etc.).

General synthetic approaches to our functionalized PEG azide derivatives (**3**)-(**7**) are shown in **Scheme 1**. The PEG starting materials range from mol. wt. 2-20K (n ~ 47-454), as determined by MALDI MS and GPC analysis. PDI's were well controlled during the PEG-OH synthesis and typically range from 1.01-1.05. Our previous mesylate synthesis required flash column chromatography (FCC). Three changes to the synthesis eliminated the need for FCC. First, the starting PEG was dried via azeotropic distillation with toluene to remove trace moisture. Second, the reactants were dried and purified via vacuum distillation; mesyl chloride from P_2O_5 and triethylamine from CaH_2. Finally, the crude reaction mixture was washed with brine-saturated 1M HCl to remove any mesylate and triethylamine salts. Precipitation of the crude product from CH_2Cl_2, Et_2O: ~1,15 yielded the pure mesylate intermediate in high yield. Displacement of the mesylate with excess sodium azide in refluxing ethanol proceeded smoothly. Extractive workup and precipitation from CH_2Cl_2, Et_2O mixtures delivered the final PEG azides (**3**)-(**7**) on multigram scales and in good overall yield.



Scheme 1. Synthesis of Azido PEG Derivatives

Monitoring of the PEG azide reaction and determination of final product purity is oftentimes problematic since TLC or HPLC analysis may fail to adequately resolve the reaction constituents and the 400 MHz ^{1}H-NMR chemical shift of the newly formed PEG-C$\underline{H}_2$-N_3 methylene protons tend to overlap with the ^{13}C satellites peaks associated with the PEG manifold (see **Figure 1**). This issue is especially pronounced with the higher molecular weight (i.e. ~ 10-20K) PEG analogs that have larger satellites. In order to overcome this limitation, we sought to identify a simple, rapid, and robust protocol that would afford products whose ^{1}H-NMR signals were distinctly different and shifted far away from the massive PEG proton manifold, typically observed at δ~3.35-3.65 ppm. To this end, we envisioned use of a click reaction to form substituted triazole derivatives.

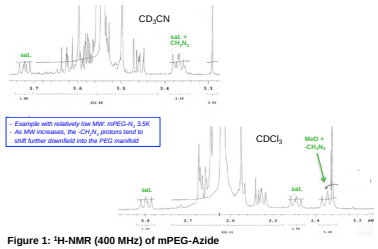


Figure 1: ^{1}H-NMR (400 MHz) of mPEG-Azide

In our model studies, we surveyed a range of solvents and conditions for copper-free click reactions with mPEG-N_3 3.5K (type **3**) with four dipolarophiles: dimethyl- and diethyl acetylene dicarboxylate (DMAD, DEBD, respectively), and diphenyl- and phenylacetylene (DPA, PA, respectively).[3] The former diesters both worked well while the latter arylalkynes did not react without catalysis (**Table 1**). We chose DEBD as the preferred dipolarophile due to its high reactivity, low cost, and elimination of the regiochemistry issue associated with use of unsymmetrical alkynes in this reaction. Note that yields are not optimized as we removed aliquots for NMR reaction monitoring.

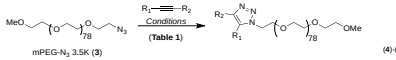


Table 1. PEG-N_3 Click Reaction Methodology Development

Rxn #	Alkyne	Solvent	Rxn Condns	Scale (mmol)	Yield (%)	NMR (CD_3CN)	Comments
1	DMAD (2x)	D_2O	RT, 12 h	0.10	ND	ND	~50% conversion but insoluble byproducts as well, pH ~4
2	DMAD (2x)	D_2O	RT -> 70°C, 2.5 h	0.10	88.0	92.5% subst'n	fastest rxn rate, but more laborious workup
3	DMAD (1.2x)	MeOH	RT, 2.5 d (~38%); 65°C, 4 h, (~93% convn)	0.10	ND	ND	integrals for click adduct are low, some ester cleavage and/or byproducts formed
4	DEBD (2x)	EtOH	reflux, 10 h (~98.5% convn)	0.10	78.5	93.6% subst'n	clean rxn
5	DEBD (2x)	EtOH	Cu wire (cat.), RT, 3 d (~52-60%); then 30°C, 4 d (~86% convn)	0.10	86.0	88.5% subst'n	EtOH and MeOH slowly react with DMAD and DEAD at RT/reflux, depleting reactants during time course of click rxn
6	DEBD (2x)	CH_3CN	RT, 15 h (15%); reflux, 4 h, (~100% convn)	0.05	81.5	92.7% subst'n	lower yield due to multiple analytical aliquots
7	DEBD (2x)	$CDCl_3$	RT, 68 h, (~34%); reflux, 32 h (~100% convn)	0.10	>91.0	95.1% subst'n	see **Figure 3** ^{1}H-NMR spectrum
8	DPA (2.2x)	CH_3CN	RT, 16 h (NR); reflux 26 h (NR)	0.10	0.0	no rxn	no rxn with DPA (uncatalyzed) in CH_3CN
9	DPA (2.2x)	EtOH	35°C, 67 h (NR); reflux, 32 h (NR)	0.05	0.0	no rxn	no rxn with DPA (uncatalyzed) in EtOH
10	PA (2x)	CH_3CN	RT, 16 h (NR); reflux 24 h (NR)	0.10	0.0	no rxn	no rxn with PA (uncatalyzed) in CH_3CN

The 400 MHz ^{1}H-NMR spectra of the resultant click adducts show several distinct new signals clearly resolved from the PEG proton manifold that can be used to quantitate azide substitution. Simple integration of all new signals and averaging (n = 3) affords the percent azide substitution (**Figure 2**, compiled in **Table 1**).

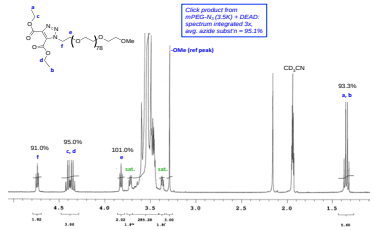


Figure 2: ^{1}H-NMR (400 MHz) of Click Adduct 2

With suitable mild reaction conditions in hand, we continued our survey using DEBD as the dipolarophile. Several additional examples of PEG azides selected from classes (**4**) to (**7**) were used and the results are shown in **Table 2**. All solid crude products were precipitated from CH_2Cl_2, Et_2O: 1,15 to 1,20. The precipitated 2K PEGs were stored at -20°C overnight prior to filtration. Click adducts from larger PEG azides (18-20K, ~1630-1850 PEG-H) are best analyzed by 600 MHz ^{1}H-NMR, which results in both greater S/N and end group integration accuracy.

(**4**)-(**7**), (R = MeO, OH, CO_2H, NH_2, BocNH)

Table 2. Further Examples of PEG-N_3 Click Reaction

Rxn #	PEG class	Azide Reactant	DEBD (eq.)	Solvent	Rxn Condns (%conversion)	Scale (mmol)	Yield (%)	NMR (CD_3CN)	Comments
11	4	N_3-PEG-NHBoc 2.1K	2.0	CH_3CN	RT, 4 d (~50%); reflux, 16 h	0.24	92.7	~98.8% subst'n	product is a slightly waxy solid
12	4	N_3-PEG-NHBoc 12.3K	2.0	CH_3CN	RT, 21 h (~trace); reflux, 27 h	0.20	88.6	~96.0% subst'n	
13	5	N_3-PEG-NH_2 2.1K	2.0	CH_3CN	reflux, 15 h	0.20	0.0	mixture	purple, mixture of click product and Michael addition byproducts
14	5	N_3-PEG-NH_2 12.5K	2.0	CH_3CN	reflux, 38 h	0.020	0.0	mixture	mixture of click product and Michael addition byproducts
15	6	N_3-$CH_2C(Me)_2$-CH_2OH	1.05	CH_3CN	RT, 71 h (~75%); reflux 8 h (~95.5%)	5.00	~Quant	>99.5% subst'n	product is a viscous oil
16	6	N_3-PEG-OH 2.1K	2.0	CH_3CN	75°C, 36 h	0.229	63.2	mixture	product and polymer present
17	6	N_3-PEG-OH 2.1K	5.0	$CHCl_3$	reflux, 24 h	0.211	67.6	>99% subst'n	
18	6	N_3-PEG-OH 2.1K	2.0	$CHCl_3$	40°C, 72 h	0.225	83.0	>99% subst'n	
19	6	N_3-PEG-OH 5K	2.0	CH_3CN	RT, 72 h (~35%); reflux 8 h (~95.5%)	0.10	89.0	~97.8% subst'n	minor amount of byproduct
20	6	N_3-PEG-OH 11.1K	2.0	CH_3CN	75°C, 36 h	0.049	72.1	mixture	product and polymer present
21	6	N_3-PEG-OH 11.1K	1.2	CH_3CN	40°C, 36 h	0.041	ND	mixture	product and polymer present
22	6	N_3-PEG-OH 11.1K	2.0	THF	60°C, 16 h	0.041	ND	mixture	mixture of side products, not polymer side product
23	6	N_3-PEG-OH 11.1K	3.0	$CHCl_3$	65°C, 48 h	0.041	85.0	>99% subst'n	
24	6	N_3-PEG-OH 11.1K	1.2	$CHCl_3$	40°C, 72 h	0.041	ND	>99% subst'n	
25	7	N_3-PEG-CO_2H 2.1K	5.0	$CHCl_3$	reflux, 24h	0.039	ND	mixture	mixture of side products, not polymer side product
26	7	N_3-PEG-CO_2H 2.1K	5.0	$CHCl_3$	RT, 36 h	0.206	84.6	>99% subst'n	
27	7	N_3-PEG-CO_2H 11.1K	5.0	$CHCl_3$	RT, 36 h	0.039	95.8	>99% subst'n	
28	3	MePEG-N3 18.6K	2.0	CH_3CN	reflux, 36 h	0.015	97.5	>98.5% subst'n	600 MHz 1H-NMR for greater S/N & integral accuracy

Procedure for PEG Azide Quantitation

1. To a 10% w/v solution of PEG-N_3 in solvent was added DEBD (1.2 - 5 eq.). The solution was stirred under argon at ambient RT or heated as described in **Tables 1** and **2**.
2. Monitor reaction progress in CD_3CN (also D_2O, $CDCl_3$) for the appearance of new, distinct triazole-derived signals and the disappearance of the PEG-C$\underline{H}_2$-N_3 methylene proton signals, which may be distinct or overlap with the right-side PEG ^{13}C satellites. Careful integration and satellite peak subtraction essential!
3. Workup: Evaporate reaction solvent, pump residue *in vacuo*, ppt'n from CH_2Cl_2, Et_2O mixtures, Et_2O washing, vacuum drying.
4. Run NMR (CD_3CN) and measure #H observed/theory for each new and/or distinct signal, take their sum, and divide by total #signals used, to afford average % substitution. Repeat twice for n = 3.

Conclusions

- Prepared a range of novel mono- and bifunctional PEG azides (**1**)-(**7**) and surveyed their uncatalyzed click reactions.
- Identified DEBD as the preferred dipolarophile and optimal reaction conditions to afford triazoles (**2**).
- ^{1}H-NMR (400-600 MHz) spectra of the resultant click adducts (**2**) show several distinct new signals that can be used to quantitate the level of azide substitution in our PEG derivatives.

- ***Scope and Limitations:***
  - Preferred solvents: acetonitrile, chloroform ($CDCl_3$), water (D_2O).
  - Alcoholic solvents (MeOH, EtOH) are generally satisfactory but slowly react with DMAD and DEBD-need to use excess dipolarophile with slower reacting PEG-N_3 substrates.
  - DPA and PA do not participate in an uncatalyzed click rxn.
  - PEG substrates containing an unprotected amino group form complex mixtures of Click and Michael addition-derived byproducts, which are inseparable.
  - In acetonitrile and THF, unprotected N_3-PEG-OH affords the desired triazole as the major product but also appear to produce a minor inseparable byproduct. Use of chloroform eliminates this issue.

References

1. Seminal review of click chemistry: (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem.* **2001**,*113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.
2. Recent reviews: Heravi, M. M.; Hamidi, H.; Zadsirjan, V. *Curr. Org. Syn.* **2014**, *11*, 647-675. Tunca, U. J. *Poly. Sci. (A)* **2014**, *52*, 3147-3165. Tang, W.; Becker, M. L. *Chem. Soc. Rev.* **2014**, *43*, 7013-7039.
3. Early studies with PEG derivatives: Garanti, L.; Molteni, G. *Tet. Lett.* **2003**, *44*, 1133.