s-Tetrazine Containing Beads for the Capture of Cancer Cells

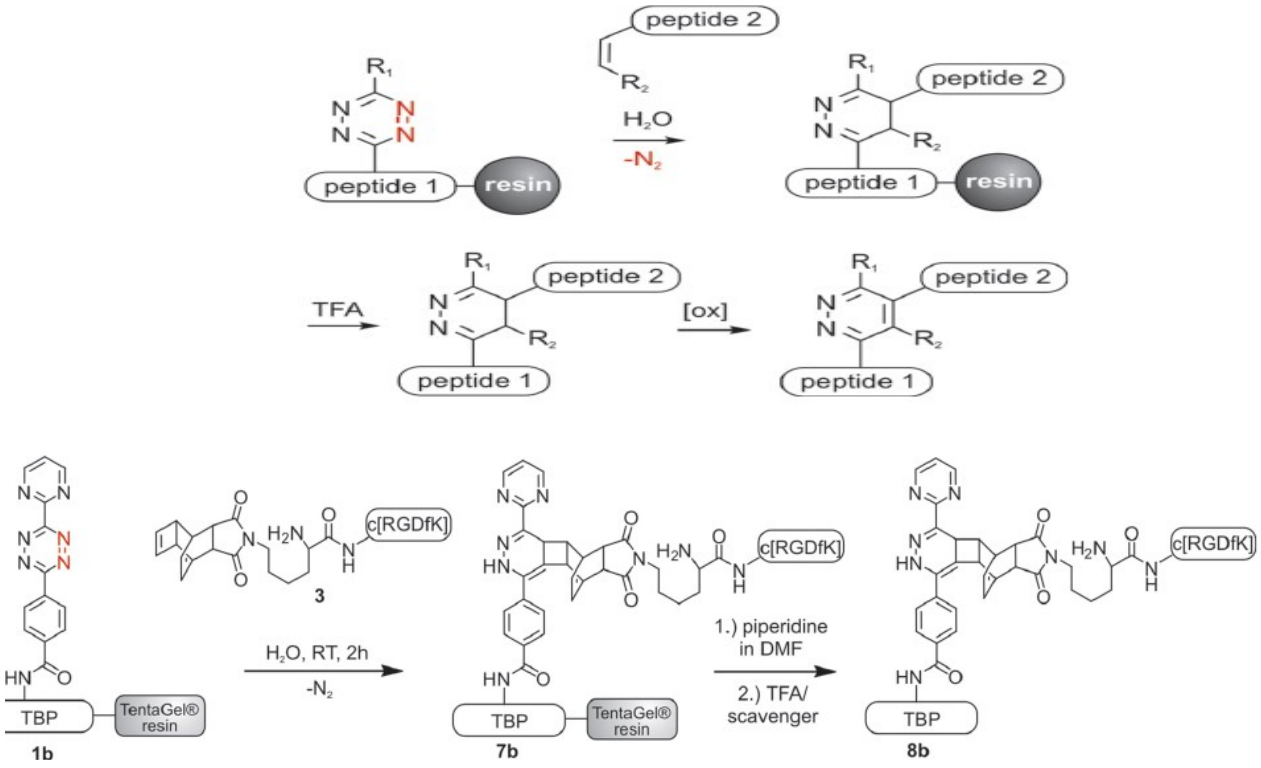
By Robert B. Login rloginconsulting.com

Can “beads” be used to remove cancer cells from blood?

The literature concerning functionalized polymeric resins(beads) promoting organic reactions is extensive; however, I have found only a few references dealing with incorporating s-tetrazines in such polymer supported resins for organic reactions.

Benaglia, M., Puglisi, A., & Cozzi, F. (2003). Polymer-supported organic catalysts. *Chemical reviews*, *103*(9), 3401-3430.

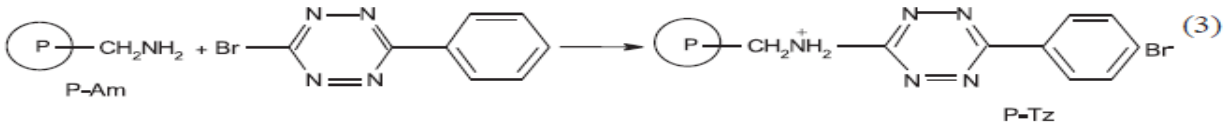
However, it has been employed in peptide synthesis where the resin is Tenta-gel



Pagel, M., Meier, R., Braun, K., Wiessler, M., & Beck-Sickinger, A. G. (2016). On-resin Diels–Alder reaction with inverse electron demand: an efficient ligation method for complex peptides with a varying spacer to optimize cell adhesion. *Organic & Biomolecular Chemistry*, *14*(21), 4809-4816.

Pagel, M. (2019). Inverse electron demand Diels–Alder (IEDDA) reactions in peptide chemistry. *Journal of Peptide Science*, *25*(1), e3141.

In many interesting studies 1,2,4,5-tetrazine was incorporated to macroporous styrene–divinylbenzene copolymer with primary amine groups (P-Am), according to the reaction (3):



(3)

1,2,4,5–tetrazine resin (P-Tz) was used for concentration and separation of noble metal ions, each at the concentration of 0.001 mol L^{-1}, in the presence of excess of Zn(II), Co(II), Cu(II), Fe(III), Ni(II) and Cd(II). The activity series for P-Tz and P-Am resins towards noble metal ions is:

$$Au(III) \sim Ir(IV) > Os(IV) > Pt(IV) > Pd(II) > Ru(III) > Rh(III)$$

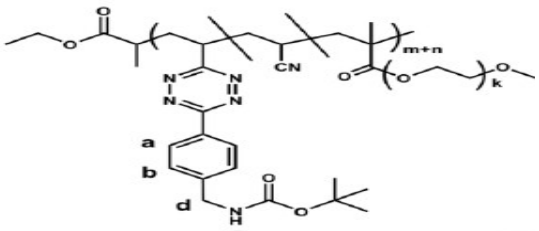
whereas for the modified polymer (P-Tz) the series changes to:

$$Pd(II) > Au(III) >> Ir(IV) > Os(IV) > Pt(IV) > Ru(III) > Rh(III)$$

High ion exchange capacity of P-Tz resin towards Pd(II) ions compared to that of P-Am resin has been explained by Grote and Topp [20] and assigned to the coordination reaction mechanism resulting in the 1:1 metal:ligand stoichiometry (Fig. 6) and to the increase in the resin hydrophobicity.

Hubicki, Z., Wawrzkiewicz, M., & Wołowicz, A. (2008). Application of ion exchange methods in recovery of Pd (II) ions–a review. *Chem. Anal.(Warsaw)*,*53*, 759-784.

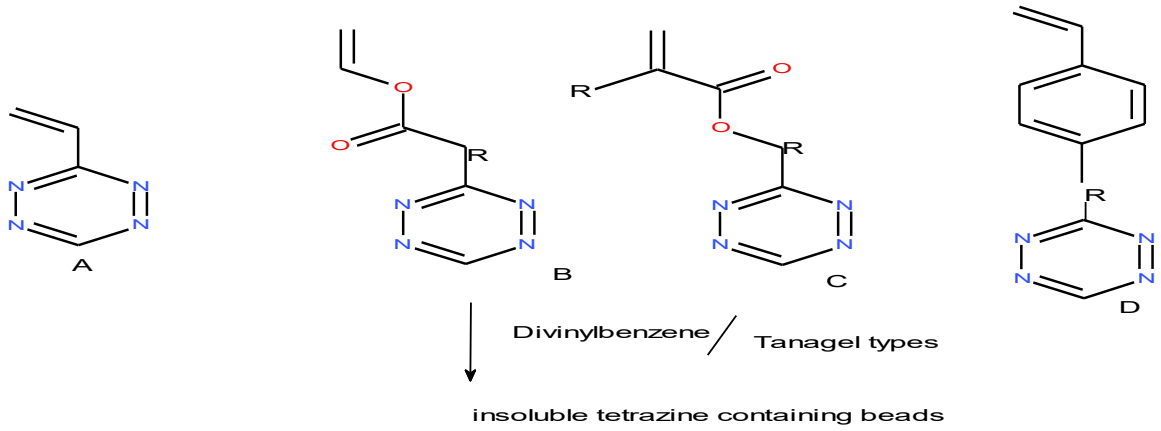
The above reference illustrates the feasibility of adding tertrazine to resins(beads).



Kara, S. S., Ateş, M. Y., Deveci, G., Cetinkaya, A., & Kahveci, M. U. (2019). Direct synthesis of tetrazine functionalities on polymer backbones. *Journal of Polymer Science Part A: Polymer Chemistry*, *57*(6), 673-680.

This reference shows the free radical copolymerization of a vinyltetrazine. However divinyltetrazine is known but it will not FR polymerize. (Pican, S., Lapinte, V., Pilard, J. F., Pasquinet, E., Beller, L., Fontaine, L., & Poullain, D. (2009). Synthesis of 3, 6-Divinyl-1, 2, 4, 5-Tetrazine, the First Member of the Elusive Vinyltetrazine Family. *Synlett*, (5), 731-734.)

Furthermore there are many references to acrylic monomers with s-tetrazine pendant groups. My idea is to find free radical polymerizable s-tetrazine monomers that can be copolymerized with divinylbenzene etc. to prepare s-tetrazine containing beads. For example:



Scheme 1: A-D examples of FR polymerizeable monomers (many others can be conceived of). The idea is that these beads can be employed to remove s-tetrazine reactive substances from solution. They could be used to purify biologic unwanted substances from say blood.

Scheme 1 is just a bare bones rendition of a rather complicated technology. For example:

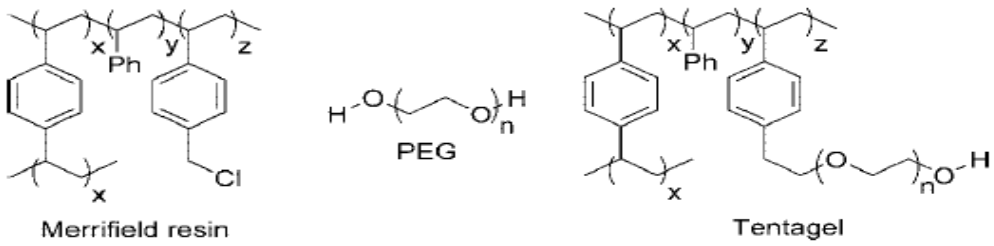
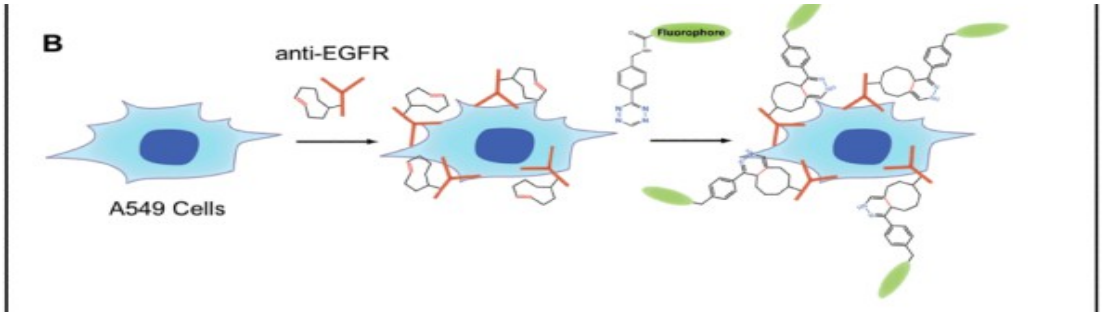


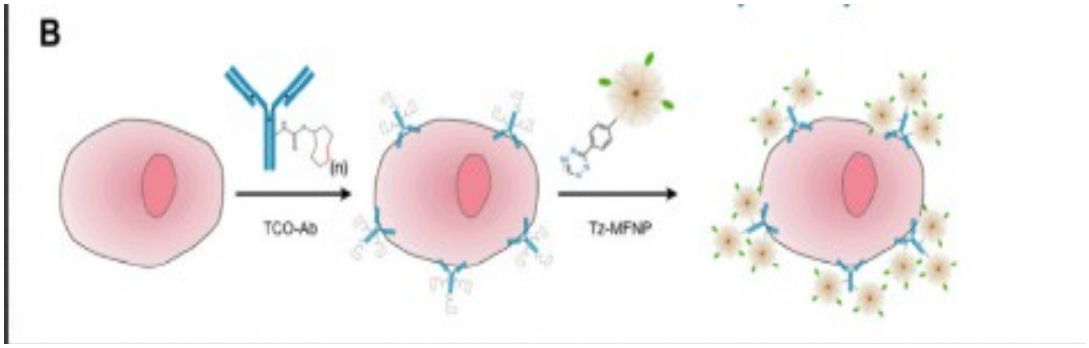
Figure 1. Structures of Merrifield resin, PEG, and Tentagel.

The scheme 1 beads could be prepared like Tenagel but with an added tetrazine monomer. The polyethylene oxide is required for my proposal as it will allow aqueous contact with blood.

Lu, J., & Toy, P. H. (2009). Organic polymer supports for synthesis and for reagent and catalyst immobilization. *Chemical reviews*, *109*(2), 815-838.

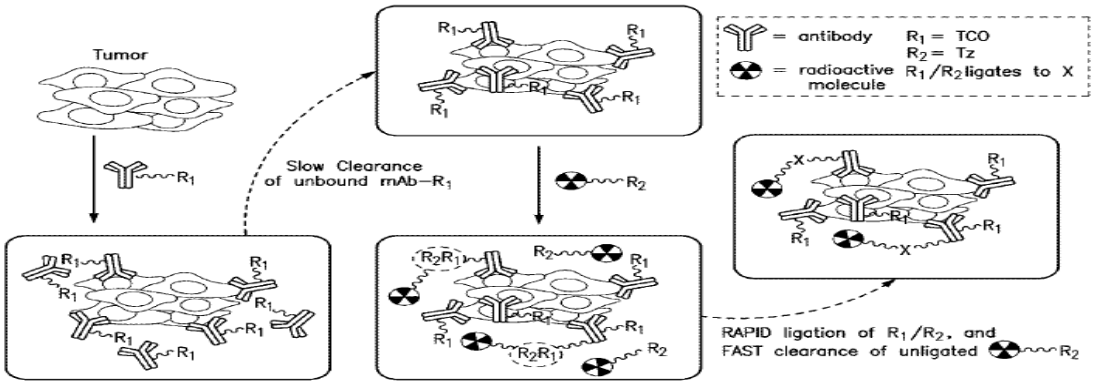
http://www.rapp-polymere.com/index.php?id=68&currency=usd3





Devaraj, N. K., & Weissleder, R. (2011). Biomedical applications of tetrazine cycloadditions. *Accounts of chemical research*, *44*(9), 816-827.

The above reference illustrates the multiple attachment of very reactive dieneophiles to a MCA(Monoclonal antibody). Their goal was to lable target cells with an easily found marker. Multiple attachments of MCA's might be valuable to firmly grasp the cancer cell in blood.



United States Patent Application Publication (10) Pub . No . : US 2019 / 0134239 A1
This patent shows how the monoclonal antibodies(MCA) functionalized with dienophiles can then be employed to be connected to a desired radio active compound in-vivo S-tetrazine derivative. The antibodies+dienophile are connected to the cancer tumor resulting in its death after the s-tetrazine ligates the radio-active compound.

ABSTRACT Pretargeting is a two-component strategy often used for tumor targeting to enhance the tumor-to-background ratio in cancer diagnosis as well as therapy. In the multistep strategy, the highly specific unlabeled monoclonal antibodies (mAbs) with the reactive site is allowed to get localized at tumor site first, and then small and fastclearing radiolabeled chelator with counter reactive site is administered which covalently attaches to mAbs via inverse electron demand Diels-Alder reaction (IEDDA). The catalyst-free IEDDA cycloaddition reaction between 1,2,4,5-tetrazines and strained alkene dienophiles aid with properties like selective bioconjugation, swift and high yielding bioorthogonal reactions are emergent in the development of radiopharmaceutical. Due to its fast pharmacokinetics, the in vivo formed radioimmunoconjugates can be imaged at earlier time points by short-lived radionuclides like ^{18}F and ^{68}Ga; it can also reduce radiation damage to the normal cells. Ultimately, this review elucidates the updated status of pretargeting based on antibodies and IEDDA for tumor diagnosis (PET and SPECT) and therapy.
Bhise, A., & Yoo, J. (2020). Pretargeting: A concept refraining traditional flaws in tumor targeting. *Journal of Radiopharmaceuticals and Molecular Probes*, *6*(1), 53-58.

To be more specific, I'm proposing that such beads(organic polymer supported resins) can be functionalized with s-tetrazines which can react with monoclonal antibodies derivatised with fast inverse electonic demand dieneophiles attached to said monoclonal antibodies in analogy with the attachment of Antibody Drug Conjugates(ADC). Furthermore, cancer cells in blood (plasma cells) can then be filtered out because the MCA has attached the very reactive dieneophile, that ready ligates to the resin(beads) which can be discarded after use and then the cancer cell free blood can be returned back to the individual. The tetrazines are usually colored but turn colorless when in pyridazine form. They will also generate nitrogen and bubble as they react. This will be telltales that the derivatised MCA is reacting with the resin beads. It is obviously possible to also put the s-tetrazine on the MCA and the dieneophile on the beads; however, I thought that the way I'm proposing would be easier from a synthesis point of view(?).

Should some cancer cells escape this filtering then the monoclonal antibody drug conjugated with an effective drug and also with the iEDDA dieneophle can then be a dual action system or both can be administed independently or together as separate compounds.

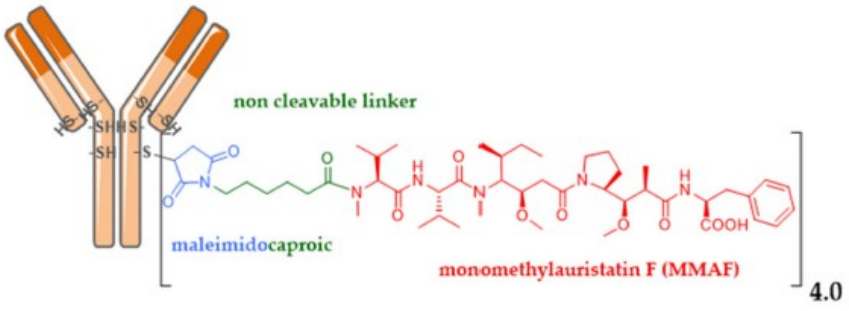


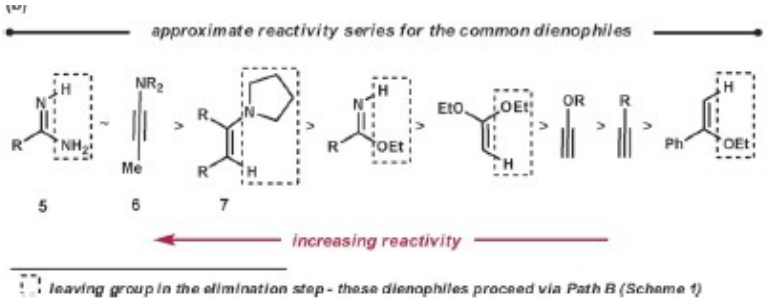
Figure 13. Formula of depatuximab mafodotin and Blenrep® (belantamab mafodotin-blmf), antibodies conjugated to MMAF via a non-cleavable linker.

On the other hand, belantamab mafodotin (GSK2857916), developed by GSK, using the same conjugation technology (maleimide + MMAF) on an afucosylated anti-BCMA IgG1 antibody (Figure 13), has successfully finished a pivotal phase II clinical study against multiple myeloma [103], for patients whose disease has progressed despite prior treatment with an immunomodulatory agent, proteasome inhibitor and anti-CD38 antibody. Following a biologics license application (BLA) filled early in 2020, Blenrep® has just been approved by the FDA as well as by the EMA, as a first-in-class anti-BCMA therapy against multiple myeloma.

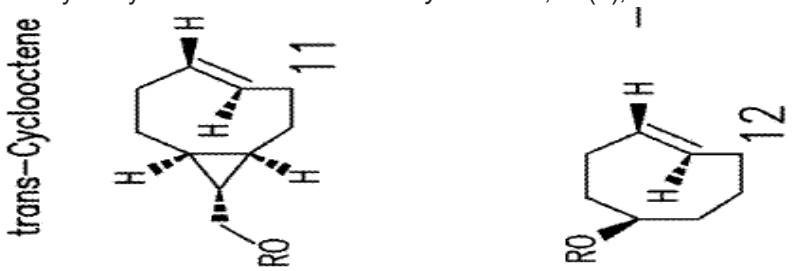
Joubert, N., Beck, A., Dumontet, C., & Denevault-Sabourin, C. (2020). Antibody–Drug Conjugates: The Last Decade. *Pharmaceuticals*, *13*(9), 245.

The above shows complicated ADC and how they are attached in this case to a MCA.

The iEDDA dieneophile could be attached by a chemical linkage to the inactive coupling section of the above ADC. It could also be another independent linkage to a free -SH site, or to a completely different MCA by one of several linkage reactions.

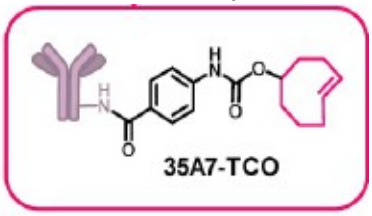


Foster, R. A., & Willis, M. C. (2013). Tandem inverse-electron-demand hetero-/retro-Diels–Alder reactions for aromatic nitrogen heterocycle synthesis. *Chemical Society Reviews*, *42*(1), 63-76.



Dieneophiles of the above type structure are usually classified as the fastest to react with the s-tetrazines. All of the above dieneophiles are non-limiting examples.

The term 'bioorthogonal' is used to describe chemical reactions that occur under physiological conditions *in vitro* and *in vivo* (pH and temperature) without interfering with biological molecules. Of the various bioorthogonal reactions that have been studied over the past two decades, the inverse-electron demand Diels-Alder cycloaddition (IEDDA) is the most rapid, with high second order rate constants ranging from 10^3 to 10^6 $M^{-1}S^{-1}$ [12,13]. The IEDDA cycloaddition occurs between a dienophile such as a *trans*-cyclooctene (TCO) and a diene like 1,2,4,5-tetrazine (Tz), leading to the formation of a covalent cycloadduct [11].



Rondon, A., Schmitt, S., Briat, A., Ty, N., Maigne, L., Quintana, M., ... & Chezal, J. M. (2019). Pretargeted radioimmunotherapy and SPECT imaging of peritoneal carcinomatosis using bioorthogonal click chemistry: probe selection and first proof-of-concept. *Theranostics*, *9*(22), 6706.

Notice that it is straight forward to attach organic chemicals to an antibody. It seems like putting a hot iEDDA dieneophile somewhere innocuous would be possible without

destroying the MCA or other monoclonal antiboodies could be derivatised with only reactive dieneophiles. The patient could then be pretreated with both types if needed. Said pretreated blood can then be filtered through s-tetrazine containing resins that I refer to as beads.

A search of Google scholar for monoclonal antibodies for MM(Multiple Myeloma) resulted in 7280 2020 hits! I am not an expert in MM biochemistry and my proposal maybe from someone not knowledgeable enough concerning this science but I feel compelled to put my proposal forward because it might be of value for blood cancers.

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