Hydrogel based drug carriers for controlled release of hydrophobic drugs and proteins
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Appendix D

The Synthesis of Heterobifunctional Linker based on
\(N\)-Succinimidyl 3-(2-Pyridyldithio) Propionate (SPDP)

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Abstract

A concise method for preparing the novel heterobifunctional linker (PDPS) with flexible lengths is reported. Its tosylated alcohol group reacts with amino groups and the 2-pyridyldisulphide structure reacts with aliphatic thiols.
Mesoporous silicas, such as MCM-41 and SBA-15 silicas, are solid materials, which are comprised of a honeycomblike porous structure with hundreds of empty channels (mesopores) that are able to absorb/encapsulate relatively large amounts of bioactive molecules. The unique properties, such as high surface area and large pore volume make them potentially suitable for various controlled release applications.\(^1\) Nowadays it is possible to make multifunctional SNPs with desired optical, chemical and, and magnetic properties all combined in one single nanostructure. Such particles are potentially useful for imaging cell, tissues, and other organs, as well as drug delivery to specific targets.\(^2\)

During the course of our studies involving the mesoporous silica nanoparticles coated with molecular valves,\(^3,4\) we sought a method for tuning the length of the well-known heterobifunctional \(N\)-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) with flexible dimensions. It was achieved by employing polyethylene glycol (PEG) derivatives which are ideal for this purpose because they are inexpensive, water soluble, and available in a variety of lengths. Hydrolysis\(^5\) of the \(N\)-hydroxysuccinimide (NHS) ester is prevented in our case by activating the alcohol group of PEG with tosylation, which is reactive to the amine functionalized silica particles, reported recently.\(^4\)

Synthesis of the 2-(2-(2-(3-(2-(pyridin-2-yl)disulfanyl) propanamido ) ethoxy ) ethoxy ) ethyl - 4 - methyl benzene sulfonate (PDPS) \(^8\) commenced with mono-amination of a triethylene glycol \(^6\) and is outlined in Scheme 1. The azidation of 2- (2- (2-chloroethoxy) ethoxy) ethanol \(^1\) was carried out in DMF with NaN\(_3\) at 150°C to give 2-(2-(2-azidoethoxy)ethoxy)ethanol \(^2\) in a quantitative yield.

![Scheme 1. Synthesis of compound 3.](image)

Subsequent reduction of the azide with Ph\(_3\)P/H\(_2\)O (Staudinger reaction) leads to the amine \(^3\) which can be further coupled with SPDP. SPDP \(^6\) was obtained by a two-step procedure as shown in Scheme 2. First 3-mercaptopropanoic acid was reacted with 2, 2’-
dipyridyldisulfide 4 by thiol-disulphide exchange in a solution of anhydrous ethanol under an acetic condition to afford 3-(2-pyridylthio)-propanoic acid 5 in 90% yield. The side reaction (5 can undergo thio-disulphide exchange with 3-mercapto propanoic acid) was almost completely suppressed by using a 2-fold molar excess of 4. This disulphide 5 was then converted into 6 by esterification with NHS using $N, N'$-dicyclohexyl carbodi-imide (DCC) in 75% yield after column purification. 

Scheme 2. Synthesis of SPDP.

$N$-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-3-(2-(pyridy-2-yl) disulfanyl) propanamide 7 was prepared by direct coupling the primary amine 3 to NHS ester 6 in DCM without any additional base with a yield of 80%. This reaction showed excellent selectivity of the NHS ester to amines than alcohols and no byproduct from the addition of unprotected alcohol was observed. Finally, the alcohol group was activated by tosylation in a good yield (85%) to be reactive towards primary amines of the silica particles.

Scheme 3. Synthesis of PDPS.
In conclusion, a simple and efficient synthesis of a novel heterobifunctional linker PDPS with flexible length was developed. Hydrolysis problems have been prevented by using tosylation instead of NHS esterification for activation. This heterobifunctional linker can find its applications in drug delivery, DNA or protein immobilization and for the study of enzymes and receptors.
References and notes

6 Triethylene glycol was taken as example here and synthesis of monoamino PEG with different lengths can be found in the following paper. Lebeau, L.; Oudet, P.; Mioskowski, C. Helv. Chim. Acta 1991, 74, 1697.
8 Data for 7: 1H NMR (400 MHz, CDCl3) δ 8.46-8.44 (1H, m), 7.72-7.64 (2H, m), 7.33 (1H, t, J=5.2 Hz), 7.11 (1H, m), 3.91 (1H, br s), 3.73 (2H, br s), 3.67-3.55 (8H, m), 3.45 (2H, q, J=5.6, J2=5.2), 3.07 (2H, t, J=6.8), 2.64 (2H, t, J=8.0) ppm; 13C NMR (100 MHz, CDCl3) δ 170.5, 159.3, 149.1, 136.8, 120.5, 119.5, 72.2, 69.8, 69.7, 69.3, 60.9, 38.9, 35.0, 34.0 ppm.
9 Data for 8: 1H NMR (400 MHz, CDCl3) δ 8.46-8.44 (1H, m), 7.80-7.77 (2H, m), 7.69-7.61 (2H, m), 7.36-7.34 (2H, m), 7.12-7.08 (1H, m), 6.85 (1H, t, J=5.6), 4.16 (2H, m), 3.68 (2H, m), 3.59-3.55 (6H, m), 3.44 (2H, q, J1=5.2, J2=5.6), 3.06 (2H, t, J=6.8), 2.61 (2H, t, J=7.2), 2.42 (3H, s) ppm; 13C NMR (100 MHz, CDCl3) δ 170.4, 159.3, 149.2, 144.7, 136.8, 132.5, 129.6, 127.5, 120.9, 120.6, 119.6, 119.4, 70.3, 69.8, 69.5, 69.0, 68.3, 39.0, 33.0, 34.0 ppm; MS m/z: 501 (M+H)+.