Synthesis of New Protection-free Symmetrically Branched Oligoglycerols that Conjugate with Target in One Step Under Mild Conditions

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Abstract

Protection-free Symmetrically Branched Oligoglycerols (BGL) can covalently connect with desired molecules to enhance their water affinity. These BGLs have no asymmetric center, and several free alcoholic groups are present on the minimum carbon backbone. To demonstrate the various properties of BGLs, there are remaining challenges to synthesize new protection-free BGL. Although previous BGLation has often required two steps to conjugate with desired targets, newly protection-free BGL can conjugate with desired targets in one step under favorable chemical conditions. In my doctoral research work, these newly synthesized protection-free BGLs has been covalently connected with desired targets in one step under mild reaction conditions to investigate their physico-chemical properties.

A thiol possessing polyols is suitable to prepare a stable and neutral water-affinitive metal surface. Also, thiol coating powerfully stops the undesired chemical reactions on the surface. Therefore, I have designed to prepare protection-free branched glyceryl trimer thiol (BGL003-SH) and branched glyceryl heptamer thiol (BGL007-SH) named as BGLated thiol. All the synthetic steps were carried out from the starting material branched glycerol trimer (BGL003) to the protection free BGLated thiol with a reasonable high chemical yield. By treating with these BGLs (BGL003-SH and BGL007-SH) on the metal surface shown a lower contact angle than the original metal surface.

BGLation protocols require pure functionalized BGL reagents, which can be synthesized by simple and efficient procedures and maintain a level of chemical reactivity with protein and peptide drugs functional groups under mild reaction conditions. Considering this idea, a new apex of protection-free branched glyceryl trimer alkoxyamine (BGL003-ONH_2), branched glyceryl heptamer alkoxyamine (BGL007-ONH_2), and branched glyceryl heptamer isothiocyanate (BGL007-NCS) were synthesized. Drug molecules that contain acyl functionality possessing carbon or hydrogen substitutions and the functionality will react with protection-free branched glyceryl alkoxyamine BGL003-ONH_2 and BGL007-ONH_2. SN-38 (7-ethyl-10-hydroxycamptothecin, an active metabolite of irinotecan) is very insoluble in water. This drug was properly conjugated with the assistance of the apex of protection-free branched glyceryl heptamer alkoxyamine (BGL007-ONH_2) *via* a diazo linker 4-formylbenzene

diazonium hexafluorophosphate (FBDP). After BGLation, hydroxyl functionality remains in SN-38, and it forms a very stable water-affinitive compound, an SN-38-BGL007 conjugate.

Nucleophilic attack of branched glyceryl heptamer isothiocyanate (BGL007-NCS) by amino group of peptide or protein forms a stable bond, which has a more water-affinity than the original peptide or protein. Branched glyceryl heptamer isothiocyanate (BGL007-NCS) will be used for the selective reaction of amino group modification to increase their water-affinity. I presume utilizing this BGL007 modified drug will be increase more water-affinity and more consumption in the human body. The adaptability (*in vitro and in vivo*) of this modified drug will be examined consecutively.

I hope that the above newly synthesized protection-free BGLated thiol will introduce a new coating method on the metal surface and the functionalization of BGL as BGLated alkoxyamines, isothiocyanates conjugation with targets will be established in a new format in the field of drug delivery systems to human to save lives.

Chapter 1. Synthesis of Symmetrically Branched Oligoglyceryl Thiols as for Metal Possessing Water-Affinitive Surface

Introduction

Thiol functionality is useful because thiols possessing a long alkyl chain generate a stable chemical bond between sulfur and zero-valent metal and molecular monolayers assembled (SAM). Attractive accessories such as necklace or earrings are often made from noble metals possessing stable zero-valence, such as gold, silver or platinum. Although such accessories often give us mental satisfaction, physical unpleasantness such as skin irritation for people skin sensitive is sometimes present at the direct contact point of skin. Such an unpleasant feel can be mainly due to the difference of surface property between metal and skin. A metal surface possessing stable zero-valence is generally water-repellent [1]. When inorganic hydroxides are intentionally increased on the surface of the metal by a particular oxidative method, the water-affinity of the metal surface is increased. Instead, the pH of the surface is often changed to be weakly acidic, probably due to the Lewis acidity of the metal oxide [2]. Direct contact with a poly-alcoholic material such as cotton gives a much more natural feel than metal, mainly because the essential component of cotton is cellulose, which has a lot of neutral aliphatic hydroxides. If such organic hydroxides are coated on the surface of noble metal, its surface can be water-affinitive with essentially neutral pH. However, it seems to be technically complicated

to paste very thin-sliced cotton. Furthermore, the resulting product may be visually unattractive and coated surface can be physically unstable.

It is desired to develop the stable coating method of neutral aliphatic hydroxides on the metal surface [3–4]. Thiol functionality can be helpful because it is well known that thiols promote a stable chemical bond with the zero-valent metal. However, to avoid the change in three-dimensional size and appearance, thiol can also be suitable because molecular monolayer assembles (SAM) [5] is created on the surface of the metal. Furthermore, thiol coating often suppresses undesired chemical reactions on the metal surface. "A thiol possessing polyols" can be suitable to prepare a stable and neutral water-affinitive surface, although very few water-affinitive thiols for coating have been reported [3–4].

In this research, I was synthesized protection-free branched glyceryl trimer thiol (BGL003-SH) (**5**) and heptamer thiol (BGL007-SH) (**10**), as shown in (**Fig. 1**). To design water affinitive thiol, "symmetrically branched oligoglycerols (BGL)" [6–7] was applied. These BGLs have no asymmetric center and several neutral hydroxides are present on the minimum numbers of the carbon skeleton. Furthermore, among previously reported thiols possessing aliphatic hydroxides [3–4], the thinnest coating width can be created with **5** and **10** because the molecular weights are much smaller than previously reported ones [3–4]. It is theoretically considered that very thin mono molecular layer was created with these water-affinitive thiols as well as coating with lipophilic thiols [8].

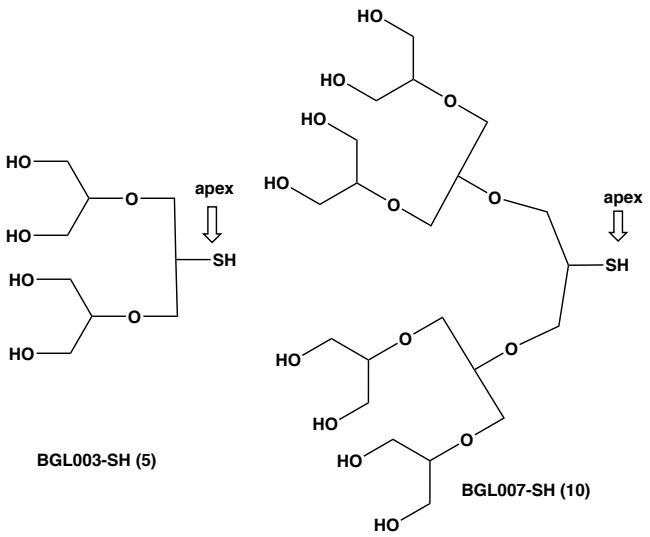


Figure 1. Symmetrically branched glyceryl trimer and heptamer with thiol functionality at apex

Property and Performance of BGL-treated silver plating material

I designed a method for preparation of silver possessing neutral water-affinitive surface by treating with protection-free branched glyceryl trimer thiol (BGL003-SH) (**5**) and protection-free branched glyceryl heptamer thiol (BGL007-SH) (**10**)**.** After treatment of a silver-plating metal piece with **5** and **10**, respectively, each contact angle was measured (**Table 1**). A measured contact angle between water and the target metal piece without treatment was 81.8˚ (entry 1). In contrast, a contact angle after treatment with **5** and **10**, was significantly smaller than the angle in entry 1 (entries 2–5). When immersing time of was longer (entries 3 *vs* 2 and entries 5 *vs* 4), a contact angle was smaller. A contact angle by **10** was smaller than by **5** (entry 4 *vs* 2 and entry 5 *vs* 3). Water-drop shape at the time of measurement was shown in (**Fig. 2**). It can be due to the difference of the number of hydroxyl groups in one molecule.

Table 1. Contact angle of BGL-treated silver plating material

Entry	Sample	Immersing Time (Sec)	Contact Angle(˚)	Standard Deviation
1	Blank	–	81.8˚	0.9
2	**5**	30	71.5˚	2.1
3	**5**	300	59.6˚	2.4
4	**10**	30	61.1˚	1.0
5	**10**	300	52.6˚	1.4

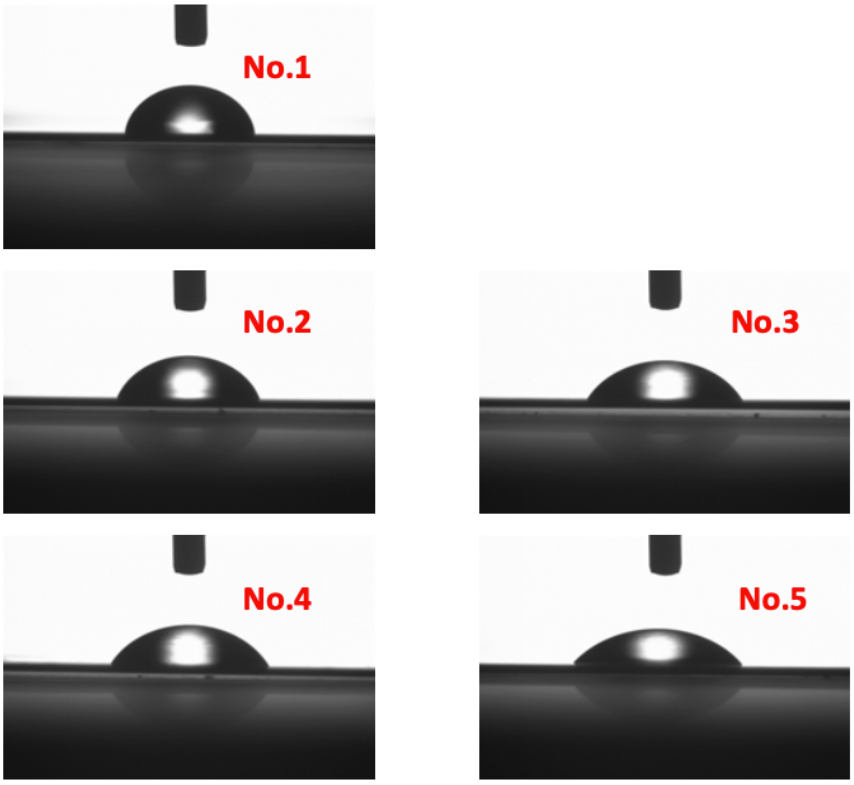
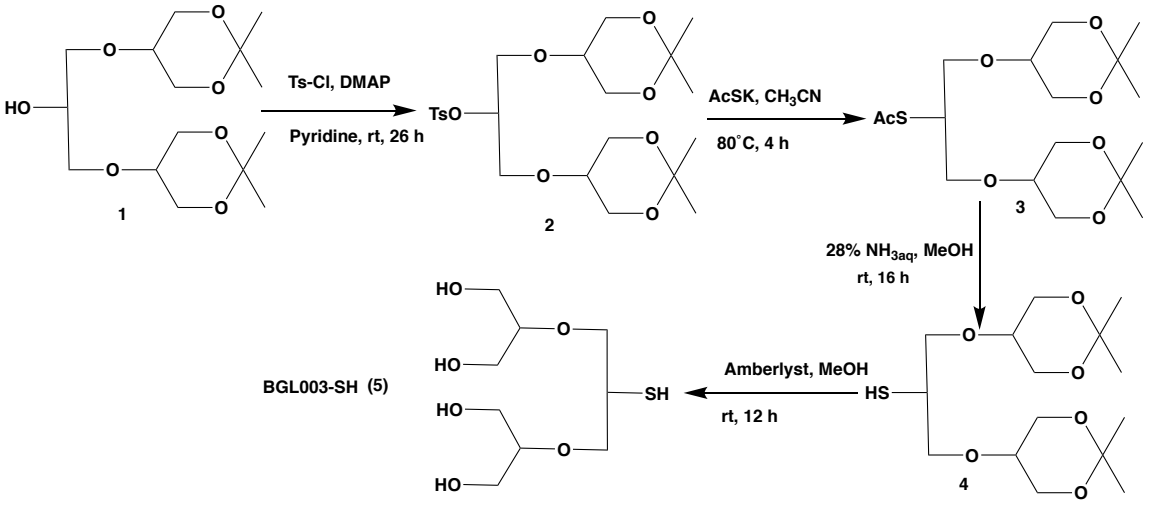


Figure 2. Water-drop shape at the time of measurement

Results and Discussion

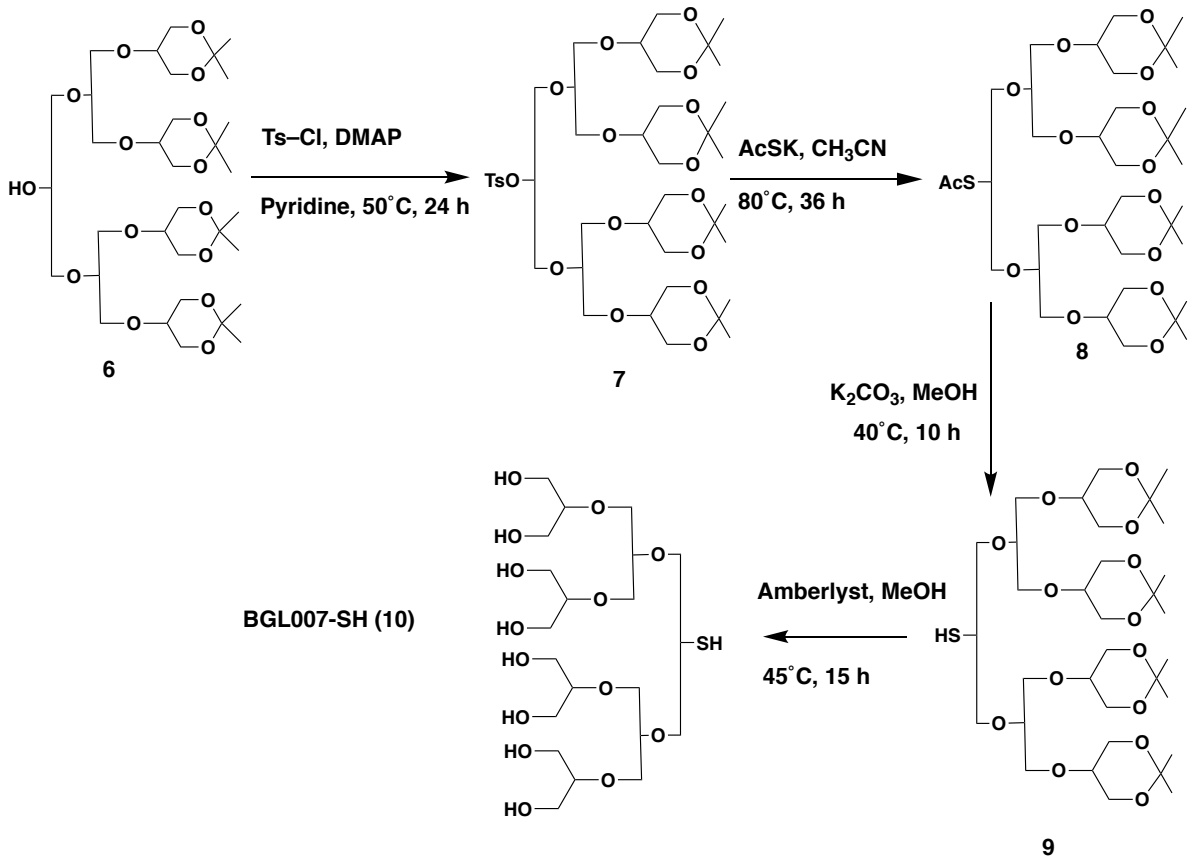
Synthesis

The detail of the synthetic procedure of compound **5** has been illustrated in **Scheme 1**. Product **1** and **2** was synthesized following the reported method [7,9]. A stirred solution of **2** with potassium thioacetate in acetonitrile to afford **3** in 93% yield. Condensation between **3** and 28% aqueous ammonia in methanol gives the corresponding intermediate compound **4** in 88% yield. Finally, compound **4** was carried out with Amberlyst®15 in methanol to afford the final product protection-free branched glyceryl trimer thiol (BGL003-SH) (**5**) in 86% yield.



Scheme 1. Synthesis of protection-free branched glyceryl trimer thiol (BGL003-SH)

The detail of the synthetic procedure of compound **10** has been illustrated in **Scheme 2**. Compound **6** was synthesized following the reported method [7]. A stirred solution of **6** with tosyl chloride, N,N-dimethyl-4-aminopyridine (DMAP) in pyridine gives the corresponding compound **7** in 57% yield. The reaction of **7** with potassium thioacetate in acetonitrile give the product **8** in 66% yield. Product **8** was stirred with potassium carbonate in methanol to afford the intermediate compound **9** in 66% yield. Finally, compound **9** was carried out with Amberlyst®15 in methanol to afford the final product protection-free branched glyceryl heptamer thiol (BGL007-SH) (**10**) in 90% yield.



Scheme 2. Synthesis of protection-free branched glyceryl heptamer thiol (BGL007-SH)

Conclusion and perspective for Chapter 1

In conclusion, synthesis of thiol possessing BGL trimer (BGL003-SH) (**5**) and heptamer (BGL007-SH) (**10**) was carried out within a few steps from the starting material branched glycerol trimer (BGL003) in high chemical yields. My resulting BGLated thiol were treated on the surface of silver plates. After treating, water-affinity of silver-plating surface was increased by immersion of BGLated thiols, because the value of a contact angle of water was decreased. In contrast, the gloss and appearance of the silver-plating was not visually changed. However, I hope that this coating method is favorable to apply various attractive accessories such as necklace or earrings as well as industrial metal materials.

Chapter 2 : Synthesis of New Protection-free Water-Affinitive Symmetrically Branched Oligoglyceryl Trimer and Heptamer

Introduction

There are several branched glyceryl trimer (BGL003), and heptamer (BGL007) with various apex groups (-OH, -NH_2) have been described [6,7] which has a very water dissolving fragment (**Fig. 3**).

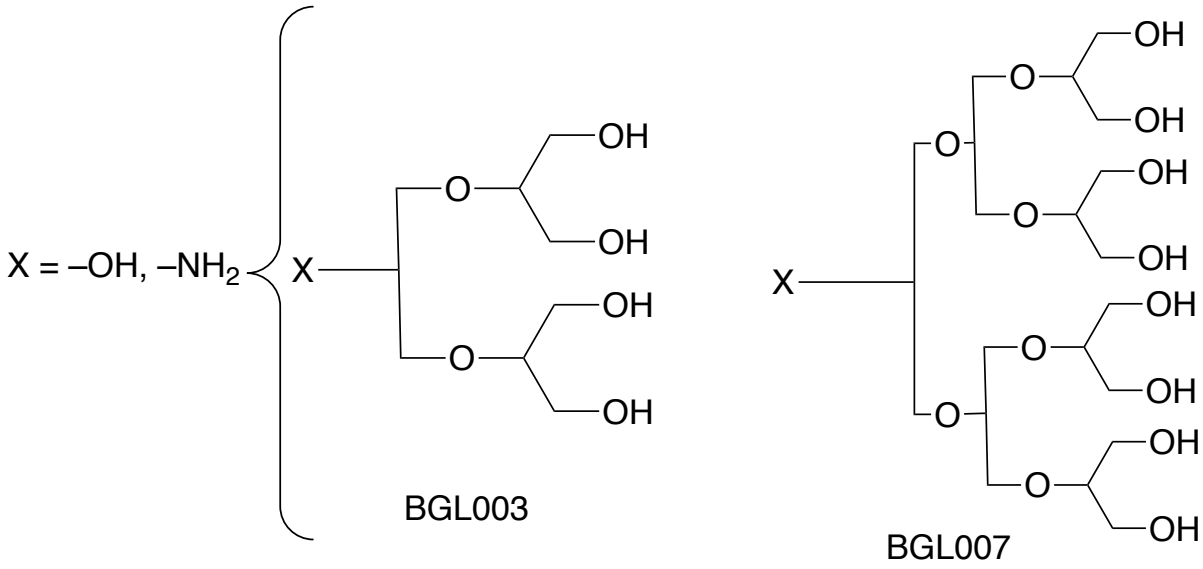


Figure 3. Originally designed symmetrically branched glyceryl trimer (BGL003) and branched glyceryl heptamer (BGL007).

BGLation technique in various drug molecules needed exceptional synthetic efficiency, linker molecules, stimulating agents, and exhaustive circumstances [9,10,11]. Protected BGLs were used in the earlier cases, and protecting groups were necessarily deprotected after BGLation in acidic circumstances. Sometimes target molecules were changed, or decomposition happened in some cases. Nowadays, this technique is not practicable in conditions in susceptible molecules like complex drugs, proteins, or peptides. Therefore, to overcome this problem, it is necessary to develop the drawbacks of the earlier BGLation method, which was shown in (**Fig. 4**).

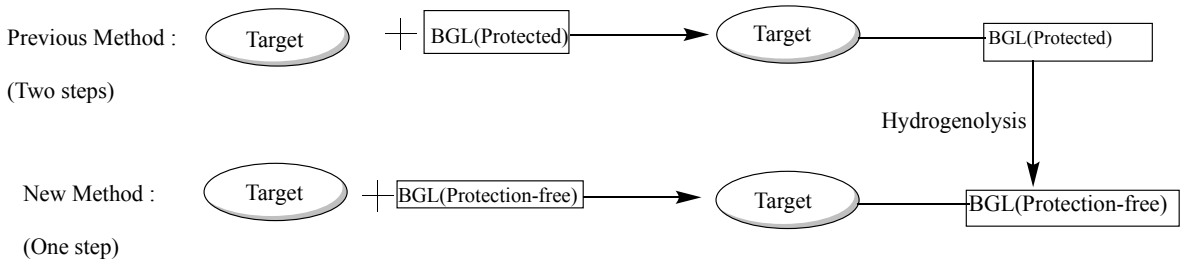
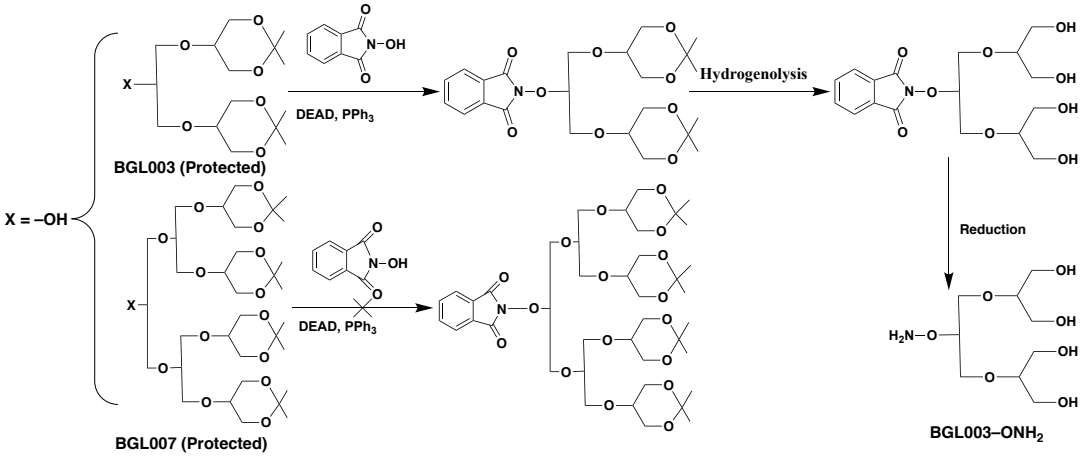


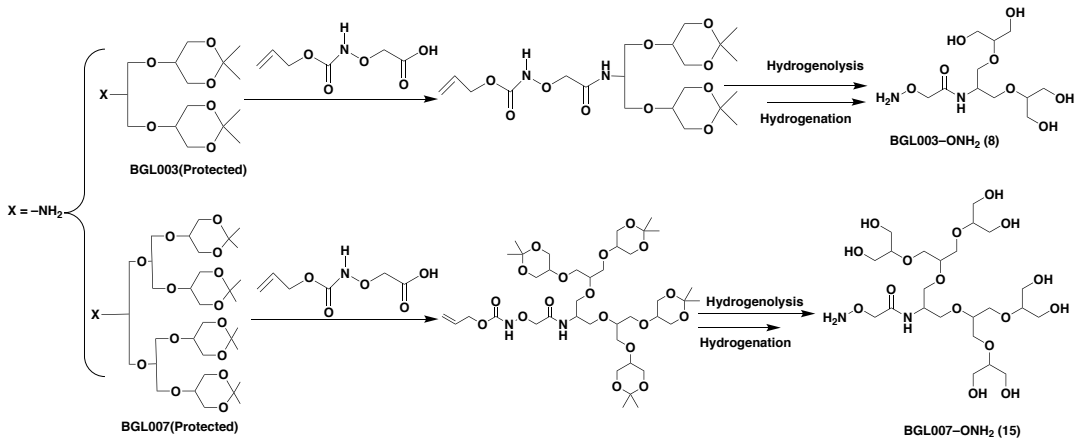
Figure 4. Previous BGLation method and new BGLation Method.

Previously a new apex of protection-free branched glyceryl trimer alkoxyamine BGL003-ONH_2 was prepared from BGL003 (Protected) by Mitsunobu reaction [12]. On the contrary, protection-free branched glyceryl heptamer BGL007-ONH_2 (**15**) was unable to be synthesized from BGL007 (Protected) by Mitsunobu reaction due to the steric hindrance of the large size of BGL007 (Protected). An additional linker was used to overcome this limitation; for the synthesize of protection-free branched glyceryl heptamer BGL007-ONH_2 (**15**). In the same way, protection-free branched glyceryl trimer alkoxyamine BGL003-ONH_2 (**8**) was also prepared (**Scheme 3**).

Previous Method (Mitsunobu Reaction):



New Method:



Scheme 3. Synthesis of branched glyceryl alkoxyamine.

On that account, herein, I have set the BGL reagents with the new apex groups, protection free branched glyceryl trimer alkoxyamine BGL003-ONH_2 (**8**), branched glyceryl heptamer alkoxyamine BGL007-ONH_2 (**15**), and branched glyceryl heptamer isothiocyanate BGL007-NCS (**17**). The detailed structure of BGL003-ONH_2 (**8**), BGL007-ONH_2 (**15**) and BGL007-NCS (**17**) have been shown here (**Fig. 5**).

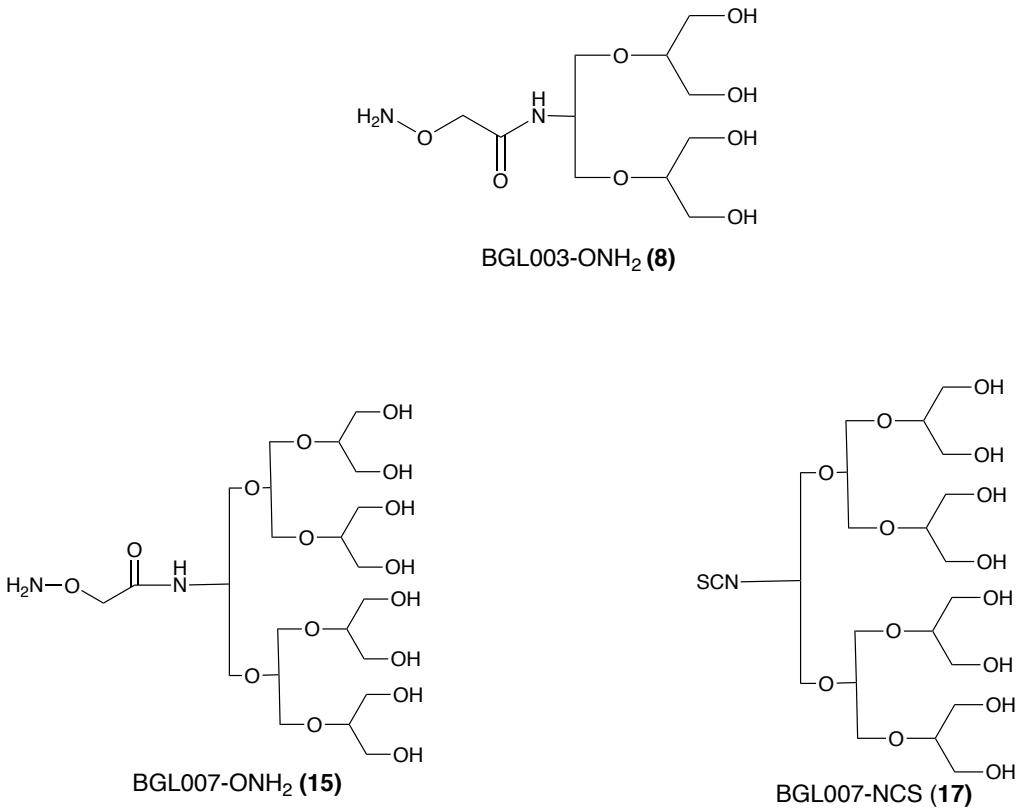


Figure 5. Branched glyceryl trimer alkoxyamine (**8**)**,** branched glyceryl heptamer alkoxyamine (**15**) and branched glyceryl heptamer isothiocyanate (**17**)

The advantages of these reagents from the previous BGL reagents are:

1. Stable and storable for a long time.
2. BGLation can be carried out in an aqueous condition without dehydrating an activating reagent.
3. BGLation condition is around pH = 7 under biologically mild temperature.
4. BGLation can be carried out at room temperature.
5. Side reactions don't happen at room temperature.
6. The compound which does not react with BGL003 can proceed with BGL007 very quickly.
7. BGL modification is shown to be an efficient method for converting water-insoluble compounds into water-soluble ones.

8. According to the number of hydroxy groups, water-affinity was found to increase geometric progress. So BGL007 derivative has more water-affinity comparing to the BGL003 derivative.
9. These new reagents are protection-free, so BGLation can proceed very quickly, remaining the original molecule unchanged. Therefore, BGLation was simply completed without any particular synthetic skill.

Lipophilic-hydrophilic balance is a pretty significant determinant of the Absorption, Distribution, Metabolism, and Excretion (ADME) properties of pharmaceuticals. For optimum ADME of a drug molecule, the use of a chemically single molecule is preferable because quality control of a single molecule is generally simple and highly reproducible. Using branched glycerol trimer (BGL003), some therapeutic molecules were converted to the corresponding water-soluble derivatives, in which water-solubility was increased 1000–5000 times [6,9,11] and molecular weight was increased only 1.5–2 times [9,11,13]. The cytotoxicity of BGL003 was evaluated and did not exhibit any significant cytotoxicity [14–15]. Consequently, BGL007 should be a safe and suitable water-solubilizing fragment for hydrophobic drug molecules.

Carbonyls (aldehydes or ketones) are reactive species and have a carbon-oxygen double bond (C=O). This binding polarity (especially in an aldehyde or ketone environment) carbon atoms exhibit electrophile and reactivity with nucleophiles such as primary amines. Alkoxyamines compounds bind to carbonyls (aldehydes or ketones), the reaction causes an oxime bond R^1–O–N=CH–R^2 (where R^1 indicates a labelling reagent and R^2 indicates a target molecule). Oximes are hydrolytically stable which possess a carbon-nitrogen double bond (C=N). The hydrolytic stability of oximes is partially due to the α-effect of the heteroatom (O) near to the sp^2 nitrogen and partially due to inductive effects that decrease the basicity of sp^2 nitrogen [16–17]. A stimulant is needed to drive the reaction quickly to completion at neutral pH.

Furthermore, in biomolecular conjugations and biomolecular labelling, the aggregations and volume of biomolecules are low enough that the biomolecules are control reagents in the reaction. This drive the use of an efficient catalyst for oxime bond formation to achieve high yields [18–19]. Here, a pair of protection-free branched glyceryl alkoxyamine BGL003-ONH_2 (**8**) and BGL007-ONH_2 (**15**) was prepared. These alkoxyamines conjugate with target molecules to form a stable oxime bond which can be reversed *in vivo* through the oxime hydrolase. When oxime hydrolase the bond, the prodrugs become active. Prodrugs that contain strongly basic amidoxime groups have displayed enhanced membrane permeability and

absorption [20]. In spite of that, an isothiocyanate is a compound with R–N=C=S, where R is a variable side chain consisting of an alkyl or aryl group. Isothiocyanates are reactive compounds, particularly concerning nucleophilic attack at the electron-deficient central carbon atom. Nucleophilic attack of isothiocyanates by amino groups of peptides or proteins forms thiourea derivatives of the structure R^1–NH–C(=S)–NHR^2 (where R^1 is alkyl or aryl group and R^2 = peptides or proteins). Here, a very reactive protection-free branched glyceryl heptamer isothiocyanate BGL007-NCS (**17**) was prepared. This isothiocyanate conjugates with peptides or proteins and takes place under mild conditions to achieve high chemical yield.

Advantages of BGLation on protein/peptide drugs

The advantages of BGLation on protein/peptide drugs are given below:

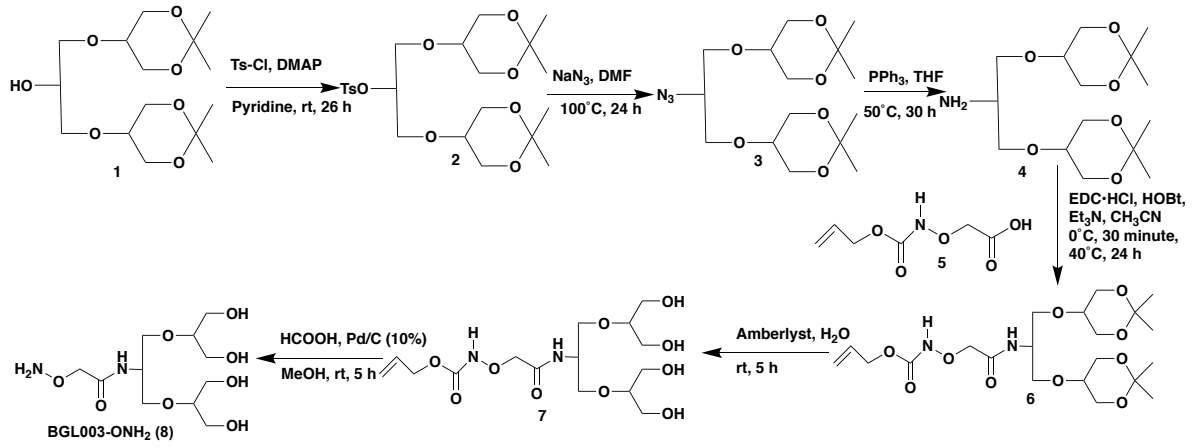
1. Reduces or prevents proteolytic degradation of enzymes
2. Increases circulating half life
3. Improves biological activity
4. Lowers its renal clearance
5. Increases molecular masses
6. Increases solubility
7. Can shield antigenic epitopes of the polypeptides
8. Cleared more slowly in the urine.

Differences between BGL003 and BGL007 derivatives

Conditions	BGL003	BGL007
Number of primary -OH groups in the BGL moiety	Less -OH groups are present	More -OH groups are present
Water affinity	Tetrahydroxylated derivative was approximately 5000 times water-ffinitive than the control compound.	Octahydroxylated derivative was at least half a million times greater than the control compound.
Temperature	Moderately to high temperature is needed to complete the reaction.	Reaction completes even in-room temperature.
Reaction time	The long time needed.	It is needed less time.
Side reactions	Side reactions maybe happen at high temperatures.	As reaction proceeds at room temperature so there is no possibility to happen any side reactions.

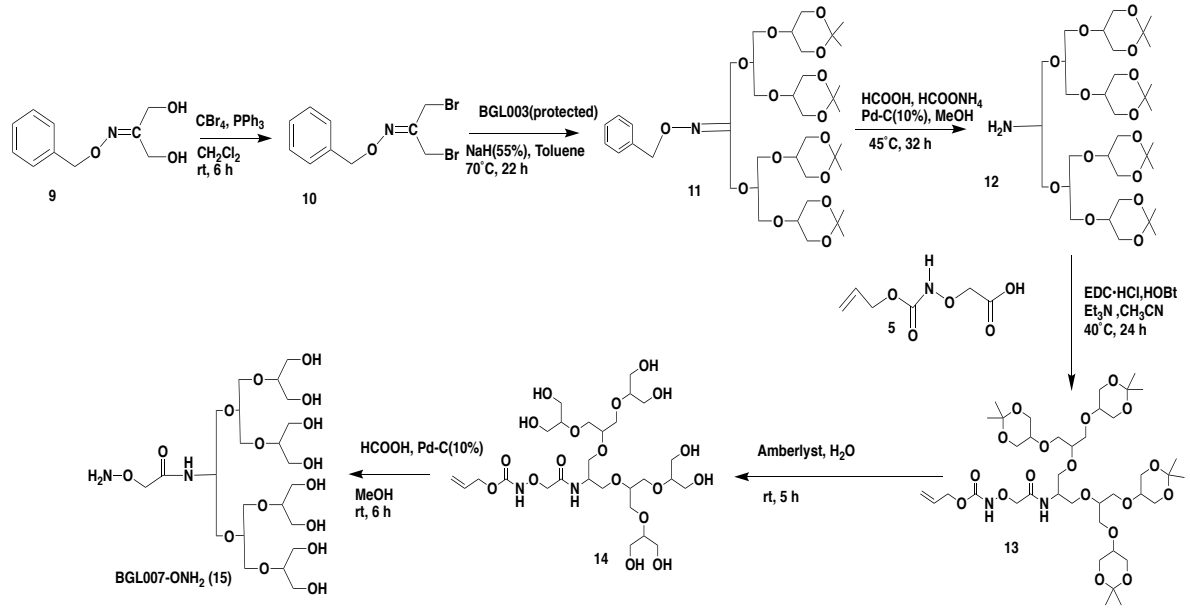
Results and Discussion

The detail of the synthetic procedure of protection-free BGL trimer alkoxyamine (BGL003-ONH_2) (**8**) has been illustrated in **Scheme 4**. Product **3** was synthesized following the reported method [9]. The reaction of **3** with triphenylphosphine in tetrahydrofuran afforded **4** in 87% yield. Condensation between **4** and 2-((((allyloxy)carbonyl)amino)oxy)acetic acid **5** [21] with EDC•HCl, HOBt, Et_3N in CH_3CN to give intermediate product **6** in 72% yield. Product **6** was stirred with Amberlyst®15 in H_2O to give **7** in 85% yield. Finally, compound **7** was carried out with Pd/C(10%), HCOOH in MeOH to afford the final product (BGL003-ONH_2) (**8**) in 66% yield.



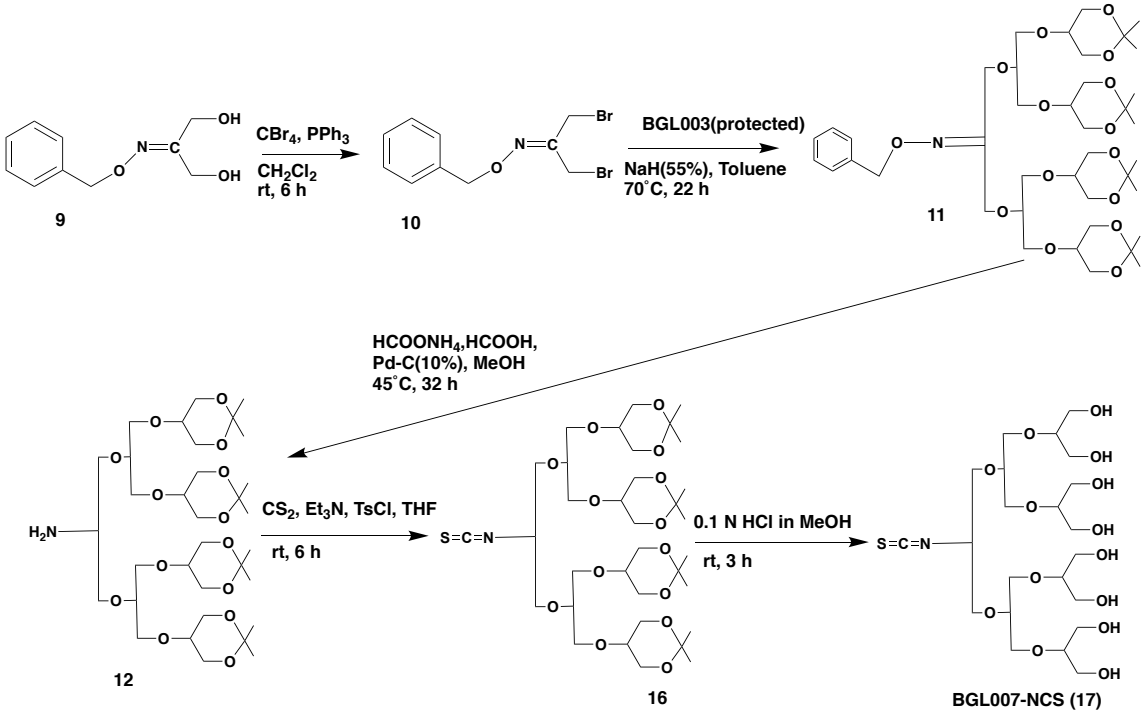
Scheme 4. Synthesis of protection-free BGL trimer alkoxyamine (BGL003-ONH_2) (**8**)

The detail of the synthetic procedure of protection-free BGL heptamer alkoxyamine (BGL007-ONH_2) (**15**) has been illustrated in **Scheme 5**. Product **9** was synthesized following the reported method [22]. The reaction of **9** with PPh_3, CBr_4 in dichloromethane afforded **10** in 79% yield. Then the reaction between **10** and BGL003(protected) in presence of toluene to give the corresponding compound **11** in 72% yield. Condensation between **11** with formic acid, ammonium formate, Pd/C(10%) in presence of MeOH afforded the compound no **12** in 61% yield. Condensation between **12** and 2-((((allyloxy)carbonyl)amino)oxy)acetic acid **5** [21] with EDC•HCl, HOBt, Et_3N in CH_3CN to give intermediate product **13** in 65% yield. Product **13** was stirred with Amberlyst®15 in H_2O to give **14** in 70% yield. Finally, compound **14** was carried out with Pd/C(10%), HCOOH in MeOH to afford the final product (BGL007-ONH_2) (**15**) in 85% yield.



Scheme 5. Synthesis of protection-free BGL heptamer alkoxyamine (BGL007-ONH_2) **15**

In **Scheme 6**, the detail of the synthetic procedure of protection-free BGL heptamer isothiocyanate (BGL007-NCS) (**17**) has been shown here. A stirred solution of **12** with carbon disulfide, tosyl chloride, triethylamine in tetrahydrofuran to afford **16** in 84% yield. Finally, compound **16** was carried out in the presence of 0.1N HCl in MeOH to give the corresponding compound protection-free BGL heptamer isothiocyanate (BGL007-NCS) (**17**).



Scheme 6. Synthesis of protection-free BGL heptamer isothiocyanate (BGL007-NCS) **17**

Conclusion and perspective for Chapter 2:

At the earlier time, when protected BGL was used with target molecules, then there was a possibility to form epimerization, racemization, cleavage of an undesired bond, or fragmentation happened at intervals, which was the main problem in the BGLation process. After a while, this problem was solved using protection-free BGL in the BGLation process. Target molecules remain unchanged at moderate temperature, controlled pH, appropriate atmospheric pressure and suitable solvents in the hydrogenolysis process, which was the limitation of the previous BGLation method. Branched glyceryl trimer alkoxyamine (BGL003-ONH_2) was synthesized from branched glycerol trimer (BGL003) by Mitsunobu reaction. The large size of branched glyceryl heptamer alkoxyamine (BGL007-ONH_2) was synthesized from branched glyceryl heptamer amine (BGL007-NH_2), including an additional linker molecule, which was the drawback of Mitsunobu reaction in the preparation of BGL007-ONH_2. The protection-free BGLation process of BGL007 has more water-affinity, carried out at room temperature, the chemical reaction conditions are mild and neutral, less time needed to complete the response comparing to BGL003. So, I firmly believe BGL007 derivatives are more water-affinitive comparing to BGL003 derivatives for converting the water-affinitive molecules. In this section, three essential protection-free reagents branched glyceryl trimer alkoxyamine BGL003-ONH_2 (**8**), branched glyceryl heptamer alkoxyamine BGL007-ONH_2 (**15**), and branched glyceryl heptamer isothiocyanate BGL007-NCS (**17**), has been developed. Drug molecules that contain acyl functionality possessing carbon or hydrogen substitutions and the functionality or adding one additional linker in drug molecules producing acyl functionality will react with protection-free branched glyceryl alkoxyamine BGL003-ONH_2 (**8**), BGL007-ONH_2 (**15**), and nucleophilic attack of branched glyceryl isothiocyanate BGL007-NCS (**17**) by amino group of peptide or protein (R^2–NH_2, where R^2 = peptide or protein) forms thiourea derivatives of the structure BGL007–NH–C(=S)–NHR^2, which will be a more water-affinitive derivative for such types of molecules.

Chapter 3 : Synthesis of SN-38-BGL Conjugate by using Symmetrically Branched Oligolyceryl Heptamer

Introduction

In this research, I planned to conjugate branched glyceryl heptamer alkoxyamine BGL007-ONH_2 (**15**) with some lipophilic molecules to enhance their water-affinity. The previous BGLation method was ordinary and required deprotection after BGLation. Herein, I have carried out deprotection process free BGLation; thus, target molecules were not changed, or no decompositions happened. A tyrosine residue is supposed to be a tremendous target for protein functionalization due to having a polar phenolic hydroxyl group that is often exposed to a protein's surface [23]. Metal-free and moderate tyrosine modification reactions [24–30] are prepossessing possibility to the commonly used lysine and cysteine modification protocols. The high abundance of lysine in a typical protein or antibody significantly complicates control of the stoichiometry and specificity of bioconjugation reactions. However, cysteines are less abundant but require reductive pre-treatment before bioconjugation. Tyrosine is less common than lysine and does not form stabilizing linkages like cysteine. Diazonium reagents have been used for selective modification of tyrosine [24,25,31] but are often not abundantly due to several limitations for the modification of proteins or antibodies. The previous reported diazonium labelling reagents are inconsistent, and just before use these reagents requires in situ preparation. Diazonium reagents are generally prepared in situ under strongly acidic conditions, which is not practicable for the diazo coupling reaction with protein. *Barbas et al*. have developed a novel bench-stable bifunctional diazonium reagent, 4-formylbenzene diazonium hexafluorophosphate (FBDP), for selective modification of tyrosine [32]. A bifunctional reagent was designed to satisfy two essential requirements: 1) improved reagent stability while maintaining high reactivity in an aqueous buffer and 2) the ability to introduce a biorthogonal functionality into the protein. The authors successfully connected the diazonium reagent (FBDP) with the tyrosine residue of N-acyl tyrosine methyl amide taken as a most miniature model of a peptide. This reagent (FBDP) was stable, maintained excellent reactivity and selectivity after more than six months of storage at 4˚C under air. The modifications by this reagent were taken place rapidly at mild and neutral conditions with a high chemical yield. The authors applied this reagent (FBDP) for tyrosine-selective modification of peptides or proteins to introduce biorthogonal aldehyde tags suitable for classical oxime and hydrazide ligations (**Fig. 6**).

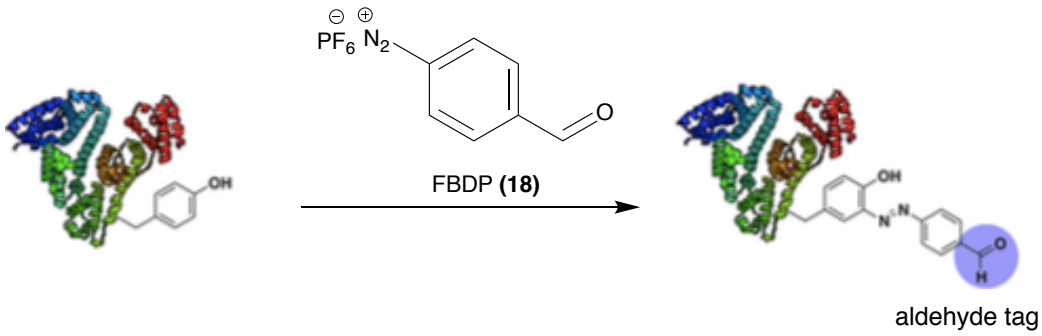
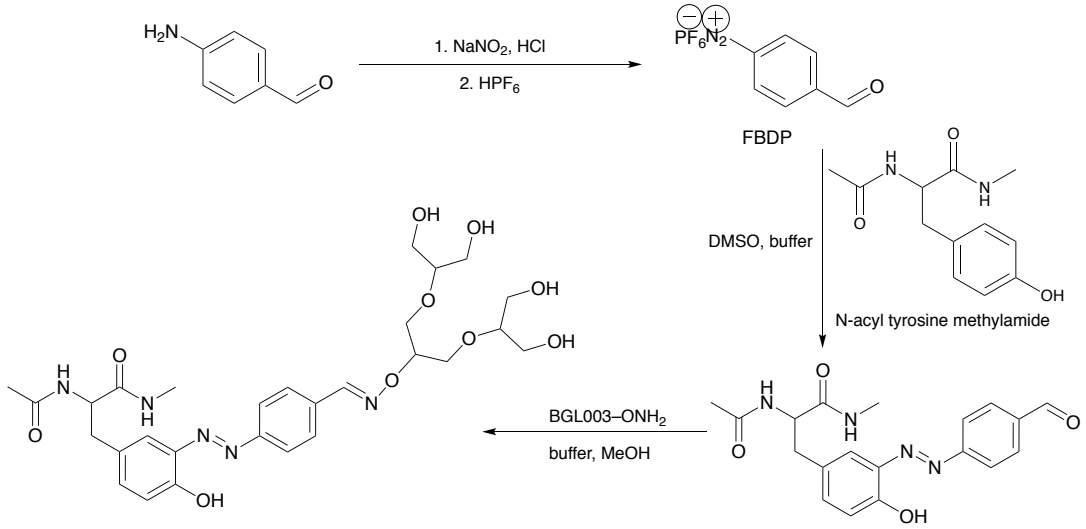


Figure 6. Selective modification of tyrosine residue in protein by FBDP.

Previously, in our research group, BGLation of *N*-acyl tyrosine methylamide with BGL003-ONH_2 *via* [(4-Formylbenzene diazonium hexafluorophosphate (FBDP)] was successfully demonstrated (**Scheme 7**) [33].



Scheme 7. Synthesis of Tyrosine peptide–BGL conjugate

Accordingly, different target was selected to screen the applicability of BGLation *via* FBDP. Herein, I designed to conjugate the new BGL reagent, protection-free branched glyceryl heptamer alkoxyamine BGL007-ONH_2 (**15**) with SN-38 (7-ethyl-10-hydroxycamptothecin) *via* this additional linker molecule FBDP (**18**). Finally, the reagent, **15** (BGL007-ONH_2) were conjugated with SN-38 *via* this additional linker molecule.

SN-38 (7-ethyl-10-hydroxycamptothecin) is an antineoplastic drug, and it is the active metabolite of irinotecan (CPT-11). It is effective against numerous malignant tumours, including lung, colorectal, gastric, lymph, cervical, and ovarian cancers [34]. SN-38 is 100–1,000 times more powerful than CPT-11 in terms of antitumor effect [35].

The lactone ring in SN-38 mainly influences the inhibition activity, which undergoes hydrolysis depending on pH [36,37]. The active lactone ring is stable at pH = 4.5 but converts to the inactive carboxylate form completely at pH 9.0 [38]. Hence, the therapeutic effect of SN-38 reduces significantly at physiological pH = (pH ≅ 7.4) [37]. In addition, it is extremely insoluble in water (11–38 μg/mL) and is poorly solubilized at 0.5% (w/w) in most physiologically consistent and pharmaceutically acceptable solvents [34,39,40]. As a result, this drug molecule cannot be administered directly and is given by slow injection into a vein. A variety of drug delivery systems have been extensively investigated, including polymeric implants [41], micelles [42–44], liposomes [45–46], polymeric conjugates [36,47], antibody conjugates [48] and nanoparticles [39,49] to emphasize the solubility of SN-38. Though these techniques enhanced the solubility but unfortunately, there were many limitations. Low encapsulation efficiency and low drug loading [40,45], poor stability, the low final yield of polymer conjugates [50] and adverse side effects are such types of case. Various studies have focused on enabling oral SN-38 delivery by synthesizing prodrugs or nanomedicines; these have shown different degrees of success [51–52]. Previously reported prodrug modifications were done through hydroxyl functionality at C20 and/ or C10 position; thus, hydroxyl functionality isn't survived (**Fig. 7**). From the literature survey, no chemical modifications were performed via diazo linker with SN-38, and no SN-38-BGL007 conjugates were known yet. Herein, I designed to connect the FBDP (**18**) reagent at the C9 position in the A-ring so that hydroxyl functionality remains survived. Finally, the reagent (BGL007-ONH_2) (**15**) was conjugated with SN-38 *via* the additional linker molecule (FBDP).

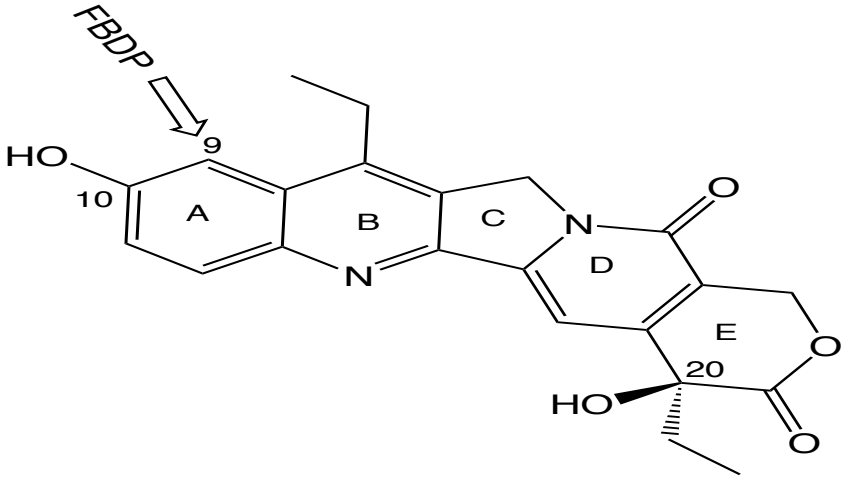
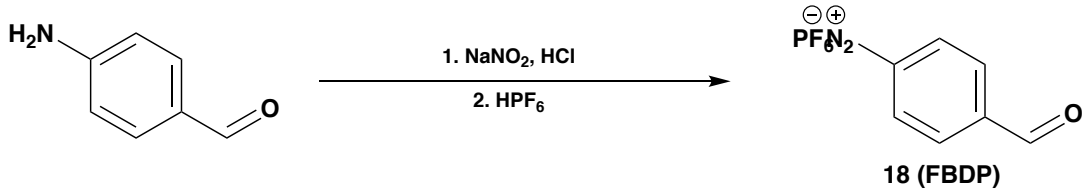


Figure 7. The linking point with FBDP is illustrated using white arrows in SN-38.

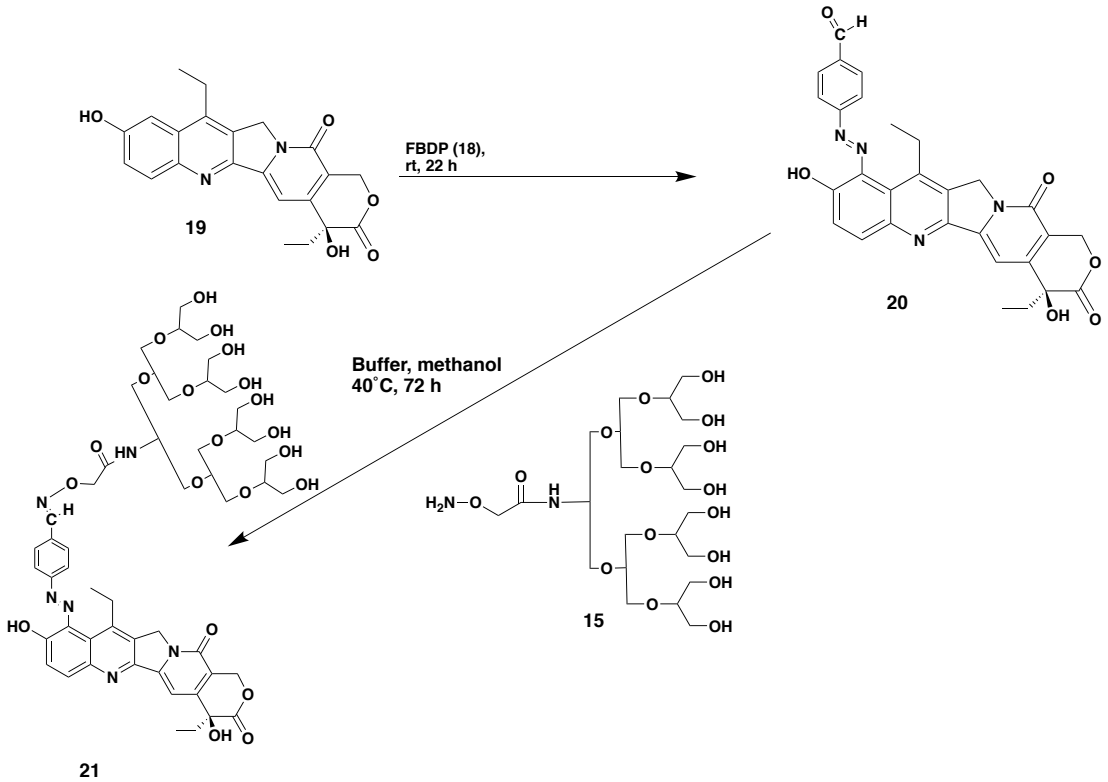
Results and Discussions:

The reagent **18** [(4-Formylbenzene diazonium hexafluorophosphate (**FBDP**)] was synthesized as the reported procedure [32] which was shown in **Scheme 8.**



Scheme 8 . Syntheis of 4-Formylbenzene diazonium hexafluorophosphate (**FBDP**) (**18**)

Herein, BGL007-ONH_2 (**15**) was used for BGLation of SN-38 *via* one additional linker molecule FBDP (**18**). In **Scheme 9**, the detail of the synthetic procedure of **21** (SN-38-BGL007 conjugate) has been presented here. The diazo coupling reaction between SN-38 (**19**) and FBDP (**18**) was carried out in the mixture of NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) and dimethyl sulfoxide (DMSO) to afford **20** in 95% yield. The reaction between **20** and **15** (BGL007-ONH_2) in NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0) and methanol afforded **21** in 73% yield. BGLation also appeared here around the physiological conditions (pH ≅ 7.4, short time, aqueous media, and mild temperature).



Scheme 9. Synthesis of SN-38-BGL007 conjugate **21**

Conclusion and perspective for Chapter 3

In this portion, one essential heptamer reagent branched glyceryl heptamer alkoxyamine (BGL007-ONH_2) has been developed. (BGL007-ONH_2) was conjugated with A-ring of SN-38 *via* one additional linker molecule (FBDP). All the synthetic steps are involved here occurred under very mild reaction conditions and found a very high chemical yield. Herein, I firmly believe these BGL-conjugated drugs will be more water-affinitive and more effective in the human body. *In vitro* and *in vivo* analyses of these modified drugs will be tested later on. I expect, in the future, more pharmaceutically and industrially important molecules might be conjugated with this BGL heptamer reagent to enhance their water affinity that will be established a new format in the field of drug delivery systems to saves human lives.

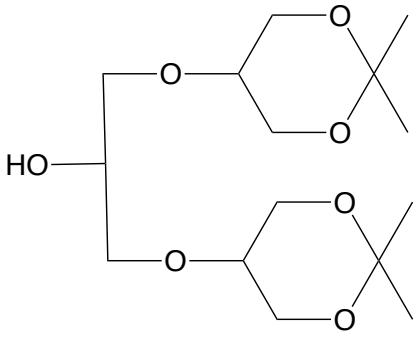
Experimental

General Information

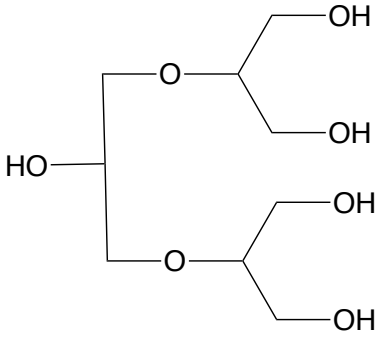
Measurement of water contact angle was carried out by Drop Master 500 available from Kyowa Interface Science Company Limited. IR spectra were measured by Nihon Bunko FT-IR 6200 spectrometer. ^1H NMR spectra were recorded by Bruker FT-NMR AV400N at 400 MHz or Bruker FT-NMR AV500 at 500 MHz, and ^{13}C NMR spectra were recorded by Bruker FT-NMR AV500 at 125 MHz in deuterated chloroform or methanol or dimethyl sulfoxide ($CDCl_3$ or CD_3OD or DMSO-d_6). Chemical shifts were indicated by δ value in ppm with tetramethylsilane as an internal standard. Multiplicities are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), brs (broaded singlet). Coupling constants, *J,* were reported in Hz (Hertz). High resolution mass spectra (HRMS) were measured by waters LCT PREMIER using Electronically Sprayed Injection–Time-of-Fight (ESI-TOF). All the reactions were carried out under nitrogen atmosphere. Reactions were monitored by thin layer chromatography of Merck Silica gel 60 F_{254} (0.25 mm) when it was applicable. Purifications were performed with Silica gel 60 N purchased from KANTO. N-hydroxyphthalimide, diethyl azodicarboxylate (DEAD), hydrazine monohydrate, SN-38, 4-aminobenzaldehyde polymer (FBDP), 3-(((ethylimino)methylene)amino)-*N*,*N*-dimethylpropan-1-aminium chloride (EDC•HCl), 1-Hydroxybenzotriazole Monohydrate (HOBt), Allyl chloroformate were purchased from TCI. 4-(Dimethylamino)pyridine (DMAP), carbon disulfide (CS_2), triphenyl phosphine (PPh_3), Carbon tetrabromide (CBr_4) hexafluorophosphoric acid (HPF_6), dehydrated tetrahydrofuran (THF) and 1,4-dioxane, dimethylformamide (DMF) and pyridine were purchased from Wako. Dimethyl sulfoxide

(DMSO) from Nacalai Tesque. Aminooxy Acetic acid/O-(Carboxymethyl)hydroxylamine hemihydrochloride (AOAA) were purchased from SIGMA-ALDRICH Company Limited. Dichloromethane (DCM) and methanol (MeOH) were distilled over calcium hydride. Pyridine was distilled over potassium hydroxide. Toluene, acetonitrile (CH_3CN) were purchased from FUJIFILM Company Limited. All other chemicals and solvents were purchased as analytical grade and used as directly unless otherwise specified.

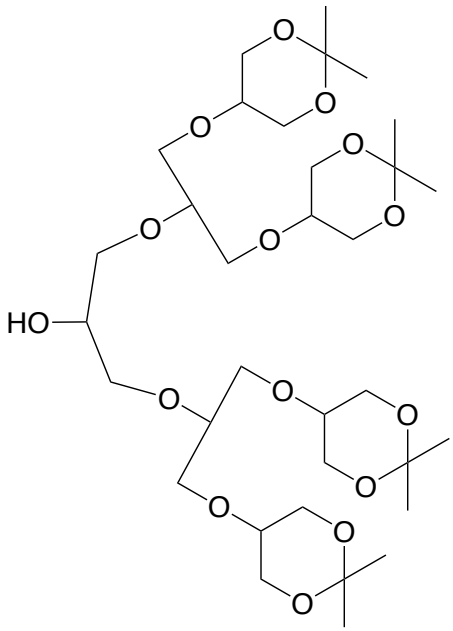
Schematic representation of glycerol trimer (BGL003) and heptamer (BGL007)



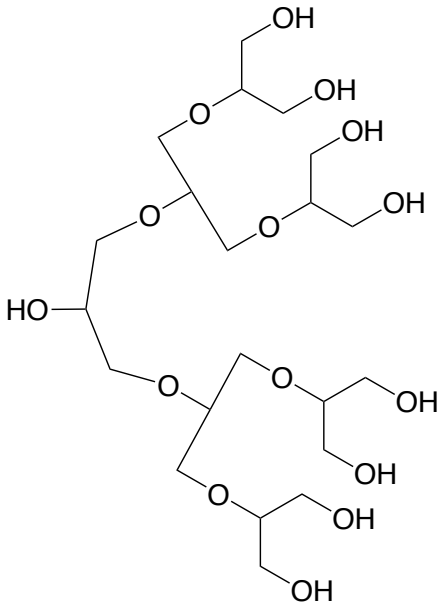
BGL003 (protected)



BGL003 (protection-free)



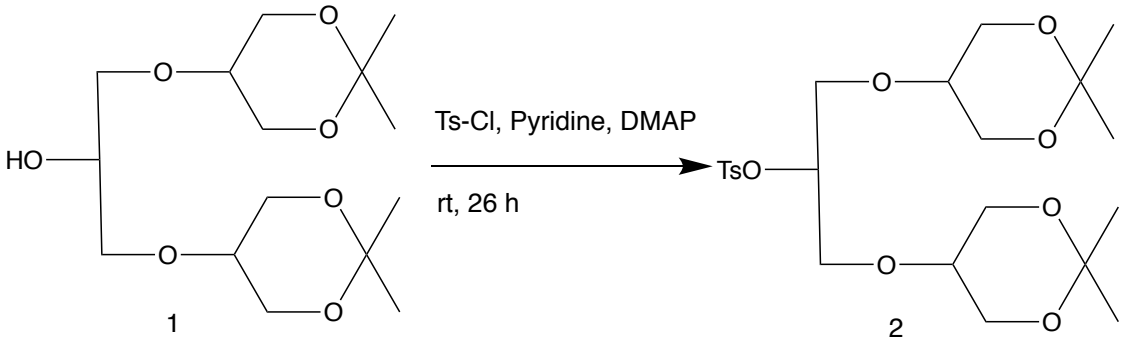
BGL007 (protected)



BGL007 (protection-free)

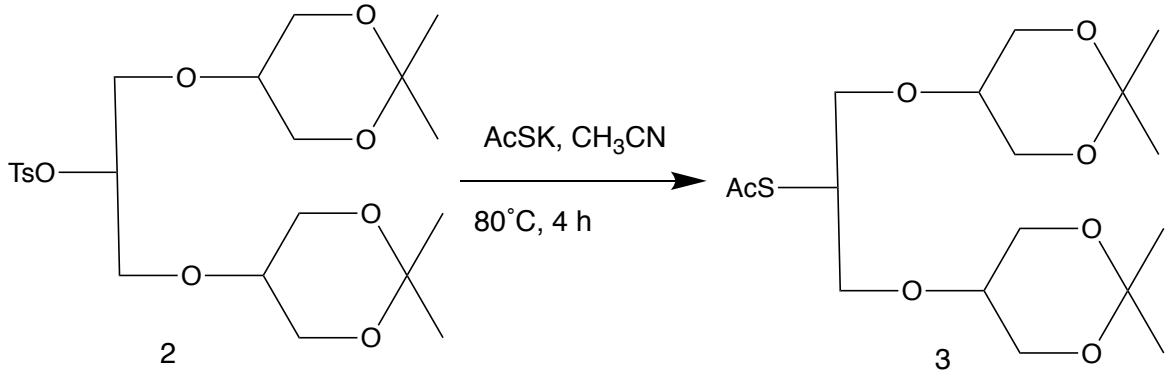
EXPERIMENTAL FOR CHAPTER 1

1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl 4-methylbenzenesulfonate (2)



To a solution of **1** [7] (2.0 g, 6.20 mmol) in pyridine (25 ml) were added DMAP (37.9 mg, 0.31 mmol) and 4-toluene sulfonyl chloride (1.78 g, 9.4 mmol), and the mixture was stirred for 26 h at room temperature. The resulting mixture was poured into 5% $KHSO_4$aq (100 mL) and extracted with ethyl acetate (80 mL × 2). The combined organic layers were washed with $NaHCO_3$aq (50 mL), brine (50 mL) and dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by silica gel column chromatography, eluting with dichloromethane/ethyl acetate (1/1) to give **2** [9] (2.94 gm, 6.19 mmol, 99%).

***S*-(1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl) ethanethioate (3)**

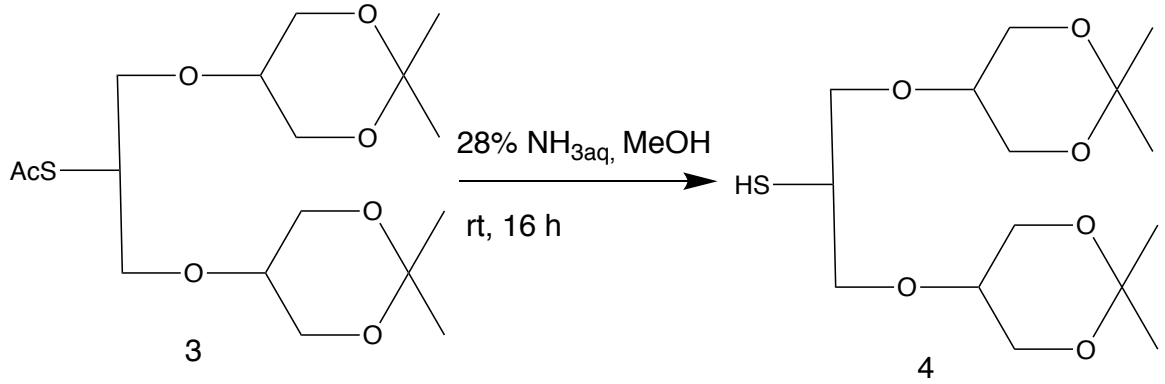


To a solution of **2** [9] (2.94 gm, 6.19 mmol) in acetonitrile (15 mL) was added potassium thioacetate (2.80 gm, 24.80 mmol), and the mixture was stirred for 4 h at 80˚C. The resulting mixture was poured into $NaHCO_3$aq (150 mL) and extracted with ethyl acetate (100 mL × 2). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel eluted by hexane/ethyl acetate (1/3) to give **3** (2.18 g, 5.76 mmol, 93%) as a red oil.

FT-IR (neat) 2992, 2873, 2360, 1693, 1456, 1373, 1251, 1199, 1088, 938, 829, 732, 634 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.97–3.93 (m, 4H, CHC**H**$_2$O), 3.83–3.77 (m, 1H, SC**H**$(CH_2)_2$),

3.75–3.69 (m, 6H, CHC**H**$_2$O), 3.64–3.60 (m, 2H, CHC**H**$_2$O), 3.50–3.44 (m, 2H, OC**H**$(CH_2)_2$), 2.33 (s, 3H, SCOC**H**$_3$), 1.43 and 1.40 (s, 12H, CC**H**$_3$; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.0 (C, C=O), 98.2 (C × 2), 70.8 (CH × 2), 67.4 (CH_2 × 2), 62.6 (CH_2 × 2), 62.5 (CH_2 × 2), 43.6 (CH), 30.6 (CH_3), 24.43 (CH_3 × 2), 22.8 (CH_3 × 2); HRMS (ESI-TOF) m/z calcd for $C_{17}H_{30}O_7SNa$ $[M+Na]^+$ 401.1610 found 401.1624.

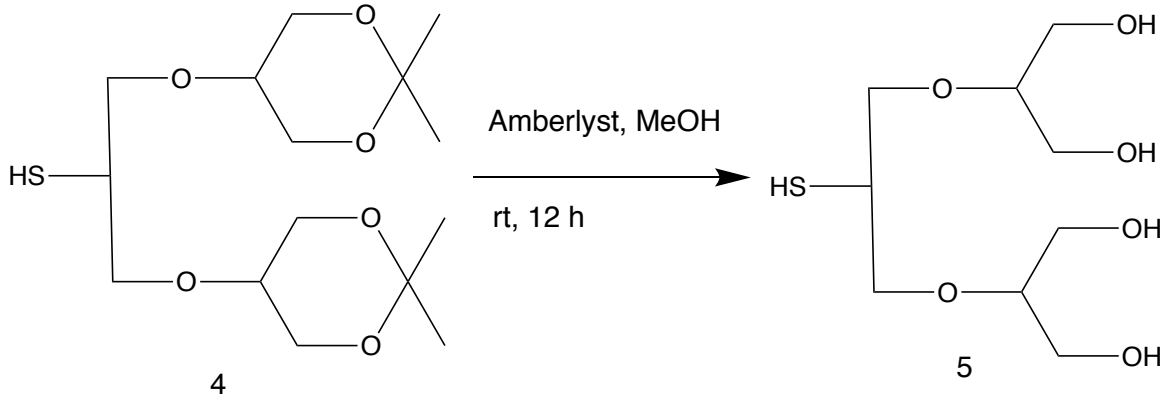
1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propane-2-thiol (**4**)



To a solution of **3** (256.7 mg, 0.68 mmol) in MeOH (2.0 ml) and 28% NH_3aq (1.0 mL) was stirred for 16 h at room temperature. The resulting mixture was poured into 5% $KHSO_4$aq (50 mL) and extracted with ethyl acetate (80 mL × 2). The combined organic layers were washed with $NaHCO_3$aq (50 mL), brine (50 mL), dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by column chromatography on silica gel eluted by hexane/ethyl acetate (1/3) to give **4** (199.8 mg, 0.59 mmol, 88%) as a red oil.

FT-IR (neat) 2991, 2872, 2360, 1456, 1373, 1252, 1228, 1199, 1089, 937, 829, 732 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.96 (dd, J = 5.5, 5.0 Hz, 4H, CHC**H**$_2$O), 3.75–3.68 (m, 8H, CHC**H**$_2$O), 3.48–3.43 (m, 2H, OC**H**$(CH_2)_2$), 3.07 (quint, J = 7.5 Hz, 1H, SC**H**$(CH_2)_2$), 1.43 and 1.40 (s, 12H, CCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 98.2 (C × 2), 71.0 (CH × 2), 67.9 (CH_2 × 2), 62.5 (CH_2 × 4), 52.4 (CH), 24.4 (CH_3 × 2), 22.8 (CH_3 × 2); HRMS (ESI-TOF) m/z calcd for $C_{15}H_{28}O_6SNa$ $[M+Na]^+$ 359.1504 found 359.1504.

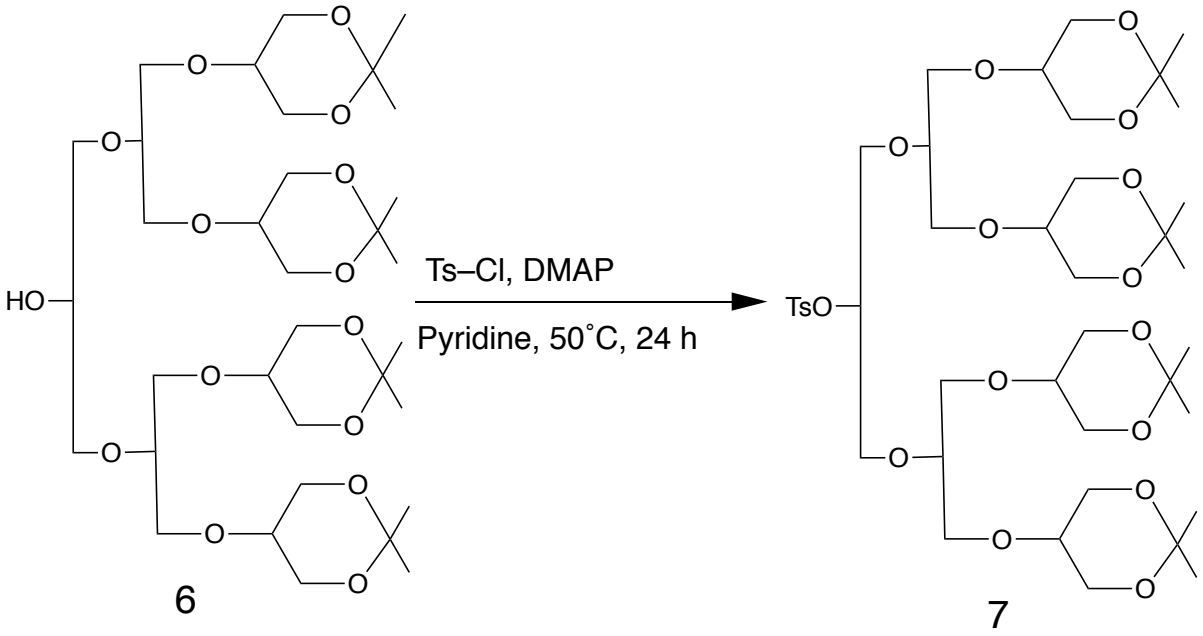
2,2'-((2-mercaptopropane-1,3-diyl)bis(oxy))bis(propane-1,3-diol) (**5**)



To a solution of **4** (165.9 mg, 0.49 mmol) in MeOH (5 ml) was added Amberlyst®15 (96.8 mg). The resulting suspension was stirred for 12 h at room temperature and filtered through filter paper. The filtrate was concentrated in *vacuo* to give **5** (108.6 mg, 0.42 mmol, 86% yield) as a red oil.

FT-IR (neat) 3370, 2932, 2878, 1652, 1465, 1407, 1347, 1120, 1050, 974, 904, 851 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 3.88 (d, J = 7.0 Hz, 4H, CHC**H$_2$**O), 3.69–3.57 (m, 8H, CHC**H$_2$**O), 3.44 (quint, J = 6.5 Hz, 2H, OC**H**$(CH_2)_2$), 3.23 (quint, J = 7.0 Hz, 1H, SC**H**$(CH_2)_2$); ^{13}C NMR (CD_3OD, 125 MHz) δ 81.6 (CH × 2), 69.1 (CH_2 × 2), 61.2 (CH_2 × 2), 61.1 (CH_2 × 2), 52.5 (CH); HRMS (ESI-TOF) m/z calcd for $C_9H_{20}O_6SNa$ $[M+Na]^+$ 279.0878 found 279.0882.

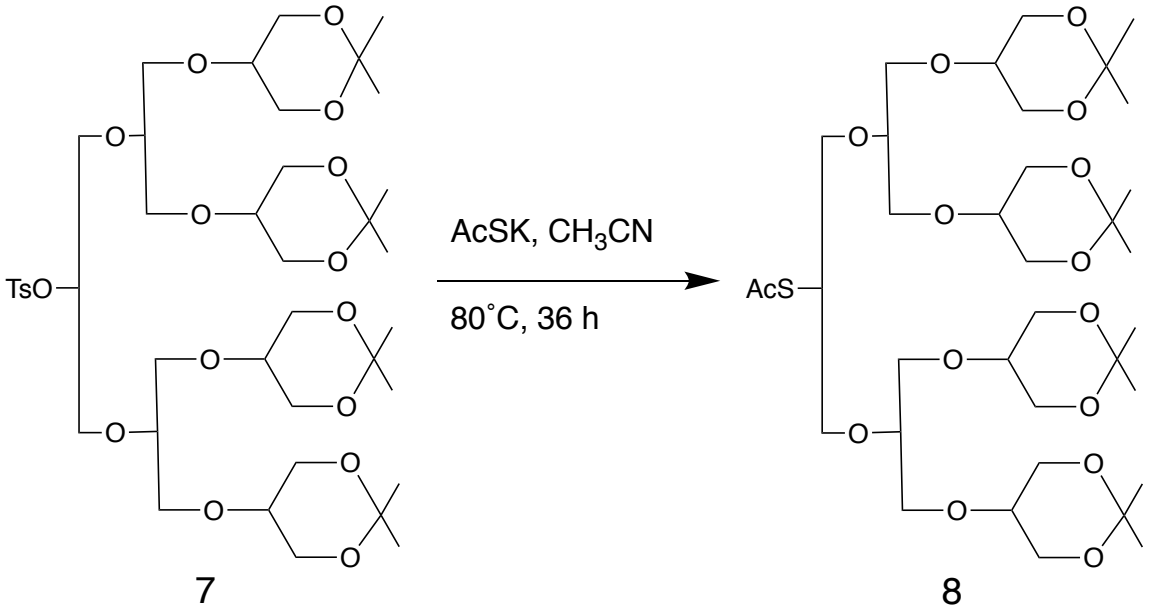
1,3-bis((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)oxy)propan-2-yl 4-methylbenzenesulfonate (**7**)



To a stirred solution of **6** [7] (100 mg, 0.14 mmol) in pyridine (0.5 mL) was added 4-dimethylaminopyridine (DMAP) (5.2 mg, 0.04 mmol) at room temperature, the solution was kept at 0˚C, added tosyl chloride (109 mg, 0.57 mmol), gradually increases the temperature up to 50˚C and the resulting solution was stirred for 24 h. The resulting reaction mixture was extracted with ethyl acetate (3 × 20 mL), followed by 5% $KHSO_4$ solution (10 mL), aqueous $NaHCO_3$ (10 mL), brine (10 mL), dried over Na_2SO_4, and the filtrate was concentrated in *vacuo*. Then the residue was purified by silica gel column chromatography, eluted with dichloromethane/acetone (2/1) to give the corresponding compound **7** (70 mg, 0.08 mmol, 57%) as a colorless oil.

FT-IR (neat) 3580, 3514, 2991, 2941, 2870, 1594, 1457, 1371, 1245, 1223, 1196, 1174, 1141, 1092, 1032, 929, 819, 732, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.81 (d, J = 10.5 Hz, 2H), 7.34 (d, J = 10.0 Hz, 2H), 4.66 (quint, J = 6.0 Hz, 1H), 3.95–3.90 (m, 8H), 3.79–3.67 (m, 12H), 3.54–3.38 (m, 14H), 2.45 (s, 3H), 1.42 and 1.40 (s, 24 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.6 (Aromatic, CH), 144.0 (Aromatic, CH), 129.7 (Aromatic, CH × 2), 128.0 (Aromatic, CH × 2), 98.2 (C × 4), 80.05 (CH), 78.9 (CH × 2), 71.02 (CH × 4), 69.0 (CH_2 × 2), 68.6 (CH_2 × 2), 68.56 (CH_2 × 2), 62.50 (CH_2 × 4), 62.47 (CH_2 × 4), 24.2 (CH_3 × 2), 24.13 (CH_3 × 2), 23.12 (CH_3 × 2), 23.06 (CH_3 × 2), 21.7 (CH_3 × 1); HRMS (ESI-TOF) *m/z* calcd for $C_{40}H_{66}NaSO_{17}$ $[M + Na]^+$ 873.3918, found 873.3914.

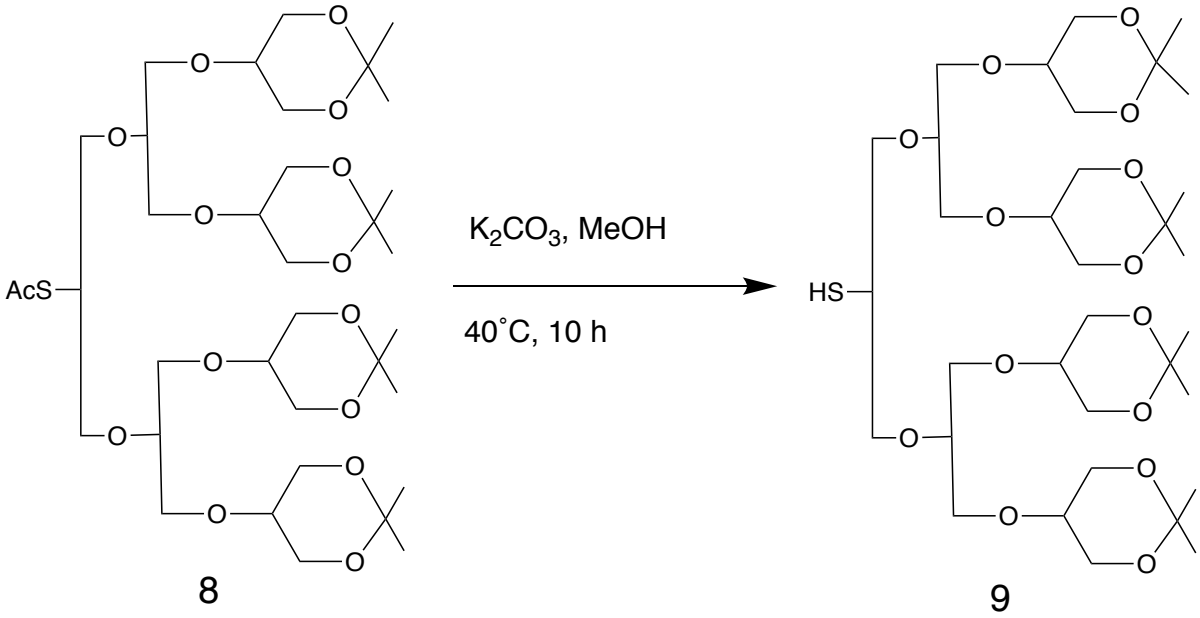
S-(1,3-bis((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)oxy)propan-2-yl) ethanethioate (**8**)



To a stirred solution of **7** (70 mg, 0.08 mmol) in acetonitrile (2 mL) was added potassium thioacetate (28.16 mg, 0.24 mmol) and the resulting solution was stirred at 80˚C for 36 h. The resulting reaction mixture was filtrate, washed with ethyl acetate (30 mL), the solvent evaporated completely and extracted with dichloromethane (3 × 20 mL), followed by adding aqueous $NaHCO_3$ (5 mL), brine (5 mL), dried over Na_2SO_4, and the filtrate was concentrated in *vacuo.* The residue was purified by silica gel column chromatography, eluted with dichloromethane/acetone (5/2) to give the corresponding compound **8** (40 mg, 0.05 mmol, 66%) as a red sticky oil.

FT-IR (neat) 3580, 3514, 2991, 2936, 2865, 1687, 1453, 1371, 1278, 1245, 1223, 1196, 1092, 1038, 934, 831, 727, 634 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.97 (dd, J = 12.0, 4.5 Hz, 8H), 3.82–3.72 (m, 13H), 3.63–3.54 (m, 10H), 3.50–3.45 (m, 4H), 2.34 (s, 3H), 1.45 and 1.42 (s, 24H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 195.13 (C, C=O), 98.18 (C × 4), 78.63 (CH × 2), 71.04 (CH × 4), 69.05 (CH_2 × 2), 68.61 (CH_2 × 2), 68.60 (CH_2 × 2), 62.59 (CH_2 × 6), 62.55 (CH_2 × 2), 44.11 (CH), 30.71 (CH_3), 24.55 (CH_3 × 2), 24.49 (CH_3 × 2), 22.75 (CH_3 × 2), 22.7 (CH_3 × 2); HRMS (ESI-TOF) *m/z* calcd for $C_{35}H_{62}NaSO_{15}$ $[M + Na]^+$ 777.3707, found 777.3735.

1,3-bis((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)oxy)propane-2-thiol (9)

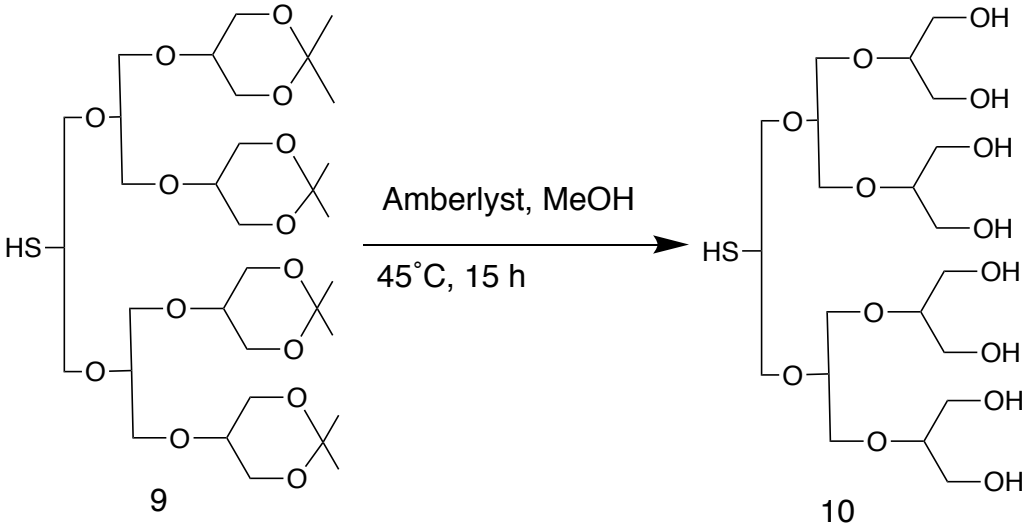


To a stirred solution of **8** (182.5 mg, 0.24 mmol) in methanol (3 mL) was added potassium carbonate (33.4 mg, 0.24 mmol), and the resulting solution was stirred at 40˚C for 10 h. The resulting reaction mixture was filtrate, the solvent evaporated completely and extracted with ethyl acetate (3 × 20 mL), dried over Na_2SO_4, and the filtrate was concentrated in *vacuo*. Then the residue was purified by silica gel column chromatography, eluted with dichloromethane/methanol (9.5/0.5) to give the corresponding compound **9** (110 mg, 0.15 mmol, 66%) as a red sticky oil.

FT-IR (neat) 3504, 2991, 2938, 2872, 1725, 1639, 1561, 1455, 1405, 1372, 1334, 1283, 1250, 1226, 1198, 1151, 1096, 1043, 987, 938, 893, 829, 755, 732, 666 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 3.95 (dd, J = 14.5, 5.0 Hz, 8H), 3.81–3.67 (m, 12H), 3.59–3.52 (m, 10H), 3.47–3.42 (m, 4H), 3.01 (quint, J = 7.5 Hz, 1H), 1.42 and 1.39 (s, 24H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 98.16 (C × 4), 78.72 (CH × 2), 71.05 (CH × 4), 69.39 (CH_2 × 2), 68.51 (CH_2 × 4), 62.55 (CH_2

× 8), 52.73 (CH), 24.46 (CH_3 × 2), 24.42 (CH_3 × 2), 22.85 (CH_3 × 2), 22.81 (CH_3 × 2); HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{61}O_{14}S$ $[M + H]^+$ 713.3782, found 713.3735.

2,2',2'',2'''-((((2-mercaptopropane-1,3-diyl)bis(oxy))bis(propane-2,1,3-triyl))tetrakis(oxy))tetrakis(propane-1,3-diol) (**10**)

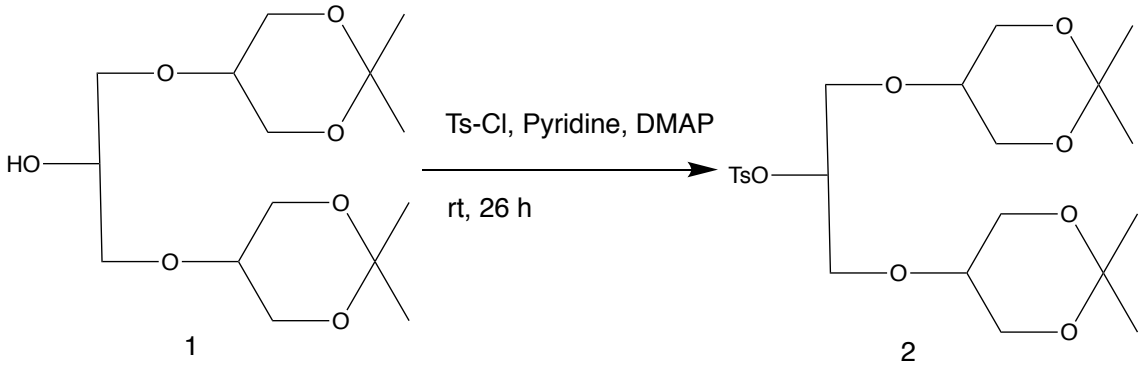


A stirred solution of **9** (110 mg, 0.15 mmol) was dissolved in methanol (2mL), and then Amberlyst®15 (110 mg) was added to this solution. The reaction mixture was stirred at 45˚C for 15 h. The resulting mixture was filtered, and the filtrate was concentrated in *vacuo* to give compound **10** (78 mg 0.14 mmol, 90% yield) as a red sticky oil.

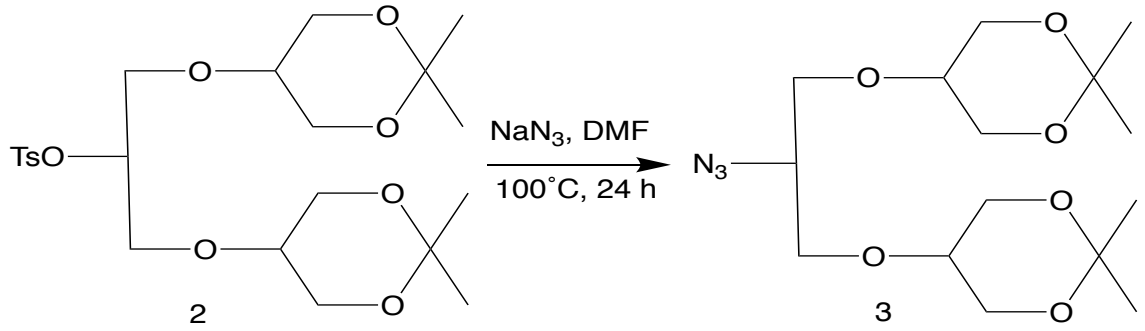
FT-IR (neat) 3377, 2925, 2878, 1644, 1457, 1408, 1343, 1256, 1108, 1043, 972, 901, 798 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 3.90–3.89 (m, 3H), 3.77–3.55 (m, 27H), 3.45 (quint, J = 6.5 Hz, 4H), 3.18 (quint, J = 6.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 81.75 (CH), 81.66 (CH × 3), 78.88 (CH), 71.08 (CH_2), 69.96 (CH), 69.38 (CH_2 × 2), 69.33 (CH_2 × 2), 69.09 (CH_2), 61.11 (CH_2 × 8), 52.27 (CH); HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{44}NaO_{14}S$ $[M + Na]^+$ 575.2349, found 575.2312.

EXPERIMENTAL FOR CHAPTER 2

5,5'-((2-azidopropane-1,3-diyl)bis(oxy))bis(2,2-dimethyl-1,3-dioxane) (3)



To a solution of **1** [7] (2.0 g, 6.20 mmol) in pyridine (25 ml) were added DMAP (37.9 mg, 0.31 mmol) and 4-toluene sulfonyl chloride (1.78 g, 9.4 mmol), and the mixture was stirred for 26 h at room temperature. The resulting mixture was poured into 5% $KHSO_4$aq (100 mL) and extracted with ethyl acetate (80 mL × 2). The combined organic layers were washed with $NaHCO_3$aq (50 mL), brine (50 mL) and dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by silica gel column chromatography, eluting with dichloromethane/ethyl acetate (1/1) to give **2** [9] (2.94 gm, 6.19 mmol, 99%).

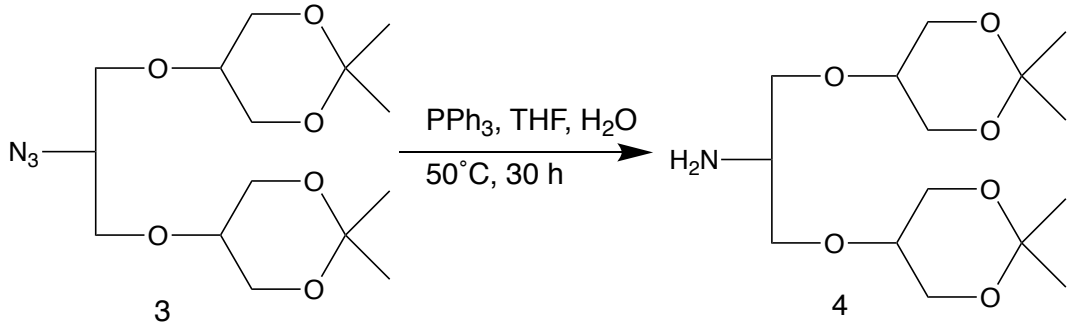


To a solution of the crude **2** [9] (470 mg) in DMF (10 mL) was added sodium azide (184 mg, 2.84 mmol), and the mixture was heated at 100˚C for 24 h. After being cooled to room temperature, the resulting mixture was poured into NaHCO3aq (100 mL), and extracted with two portions of ethyl acetate (100 mL × 2). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated in *vacuo*. The residue was purified by silica gel column chromatography eluted with hexane/ethyl acetate (1/1) to afford **3** [9] (309 mg, 0.896 mmol, 95% yield).

FT-IR (neat): 3585, 2991, 2873, 2360, 2100, 1652, 1455, 1373, 1338, 1251, 1228, 1199, 1153, 1099, 1043, 997, 939, 829, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 4.0–3.95 (m, 5H), 3.78-3.56 (m, 9H), 3.50–3.45 (m, 2H), 1.44 (s, 6H), 1.41 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ

97.9 (C × 2), 70.9 (CH × 2), 68.1 (CH_2 × 2), 62.0 (CH_2 × 4), 60.2 (CH), 23.6 (CH_3 × 2), 22.7 (CH_3 × 2); HRMS (ESI-TOF) m/z calcd for $C_{15}H_{27}N_3O_6Na$ $[M + Na]^+$ 368.1798, found 368.1802.

1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-amine (4)

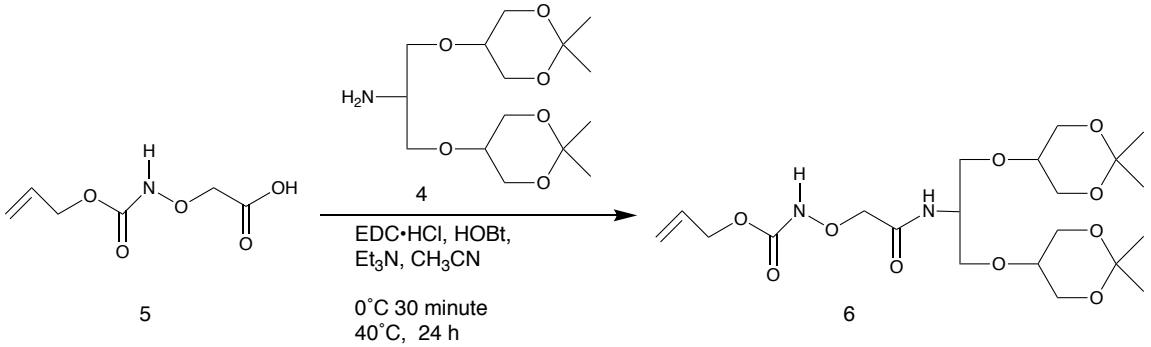


3 4

To a solution of **3** [9] (1.0 gm, 2.90 mmol) in tetrahydrofuran (THF) (7.2 mL) was added triphenylphosphine (PPh_3) (1.52 gm, 5.79 mmol) followed by water (10 drops) at room temperature and the reaction mixture was stirred at 50˚C for 30 h. After 30 h, the reaction mixture was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane/methanol (4/1) as eluent to give **4** (805 mg, 2.52 mmol, 87%) a white solid.

FT-IR (neat): 3429, 2995, 2944, 2873, 1638, 1469, 1377, 1255, 1200, 1152, 1117, 1085, 937, 825, 734, 622 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.97 (dd, J = 12.0, 4.0 Hz, 4H), 3.74 (dd, J = 12.0, 6.0 Hz, 4H), 3.52 (dd, J = 9.4, 4.8 Hz, 2H), 3.45–3.36 (m, 4H), 3.13 (quint, J = 5.3 Hz, 1H), 1.43 and 1.41 (s, 12 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 98.3 (C × 2), 71.12 (CH_2 × 2), 70.9 (CH × 2), 62.6 (CH_2 × 4), 51.12 (CH), 24.12 (CH_3 × 2) and 23.14 (CH_3 × 2); HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{29}NO_6Na$ $[M + Na]^+$ 342.1893, found 342.1927.

allyl (2-((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)amino)-2-oxoethoxy)carbamate (6)

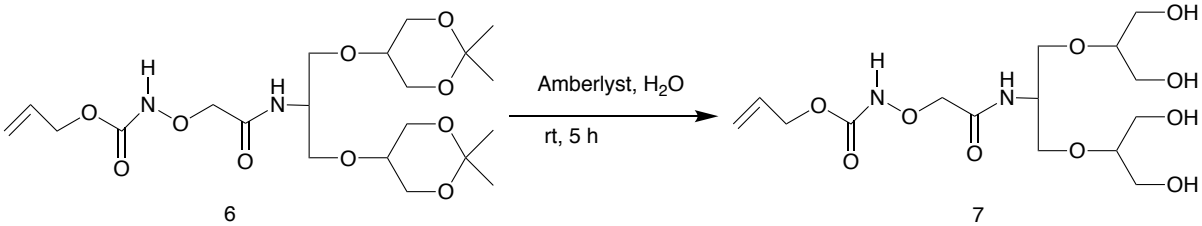


5 6

To a solution of **5** [21] (50.9 mg, 0.29 mmol) in acetonitrile (CH_3CN) (3 mL) was cooled at 0˚C. The reaction was stirred at 30 minutes, and then HOBt (57.9 mg, 0.37 mmol), EDC•HCl (72.5 mg, 0.37 mmol) was added to this solution, and the suspension was stirred for about 30 minutes. The reaction mixture was gradually warm to room temperature and then compound **4** (93 mg, 0.29 mmol), triethylamine (121 µL,B 0.87 mmol) was added. The mixture was stirred at 40˚C for 24 h. The resulting crude mixture was extracted with ethyl acetate (60 mL × 3), dried over sodium sulfate (Na_2SO_4). After filtration, the solvent was evaporated under reduced pressure, and the reaction crude was purified by column chromatography on silica gel using dichloromethane/methanol (5/1) to give the corresponding compound **6** (100 mg, 0.20 mmol, 72 %) as a yellowish sticky oil.

FT-IR (neat) 3285, 2991, 2941, 2876, 1796, 1736, 1665, 1534, 1453, 1371, 1338, 1245, 1190, 1098, 1038, 989, 929, 825, 738 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.96–5.89 (m, 1H), 5.37–5.27 (dd, J_{trans}= 17, 1.0 Hz, J_{cis}= 10.5 Hz, 2H), 4.66 (d, J = 6.0 Hz, 2H), 4.39 (s, 2H), 4.30–4.26 (m, 1H), 4.0 (dd, J = 11.5, 3.5 Hz ,4H), 3.81 (dd, J = 5.0 Hz, 4.5 Hz, 4H), 3.72–3.69 (dd, J = 9.5, 4.0 Hz, 2H), 3.59–3.56 (dd, J = 9.0, 3.0 Hz, 2H), 3.42 (quint, J = 3.5 Hz, 2H) and 1.44 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.46 (C, C=O), 157.91 (C, C=O), 131.62 (CH), 118.82 (CH_2), 98.35 (C × 2), 75.91 (CH_2), 70.5 (CH × 2), 66.69 (CH_2), 66.43 (CH_2 × 2), 62.75 (CH_2 × 2), 61.88 (CH_2 × 2), 48.33 (CH), 24.22 (CH_3 × 2) and 22.95 (CH_3 × 2); HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{37}N_2O_{10}$ $[M + H]^+$ 477.2448, found 477.2468.

allyl (2-((1,3-bis((1,3-dihydroxypropan-2-yl)oxy)propan-2-yl)amino)-2-oxoethoxy)carbamate (7)

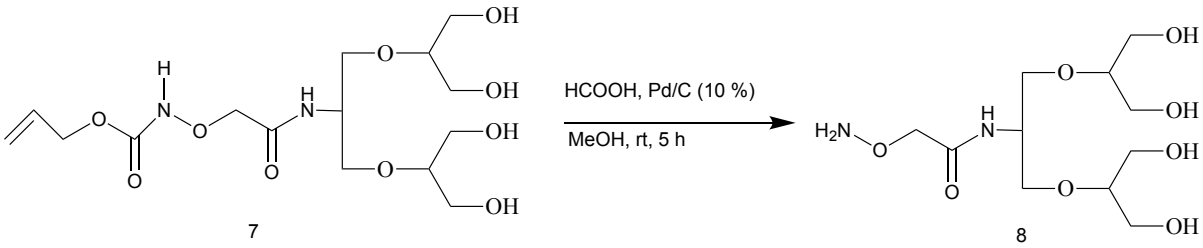


6 7

To a stirred solution of **6** (84.5 mg, 0.17 mmol) was dissolved in H_2O (2 mL) and then Amberlyst®15 (84.5 mg) was added to this solution. The reaction mixture was stirred at room temperature for 5 h. The resulting mixture was filtered, and the solvent was evaporated under reduced pressure to give the corresponding compound **7** (60 mg, 0.15 mmol, 85% yield) as a yellowish sticky oil.

FT-IR (neat) 3345, 2936, 2876, 2200, 1790, 1725, 1654, 1545, 1463, 1420, 1338, 1256, 1125, 1043, 994, 940, 858, 771 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.00–5.90 (m, 1H), 5.36–5.23 (dd, J_{trans}= 21.5, 2.0 Hz , J_{cis}= 13.0 Hz, 2H), 4.64 (d, J = 7.0 Hz, 2H), 4.32 (s, 2H), 4.21 (quint, 1H), 3.79 (dd, J = 12.0, 6.5 Hz, 2H), 3.72–3.57 (m, 10H), 3.44 (quint, J = 6.5 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ169.81 (C, C=O), 153.89 (C, C=O), 132.06 (CH), 117.21 (CH_2), 81.7 (CH × 2), 75.17 (CH_2), 68.12 (CH_2 × 2), 66.07 (CH_2), 61.15 (CH_2 × 2), 60.98 (CH_2 × 2), 49.6 (CH); HRMS (ESI-TOF) *m/z* calcd for $C_{15}H_{29}N_2O_{10}$ $[M + H]^+$ 397.1822 , found 397.1834.

2-(aminooxy)-N-(1,3-bis((1,3-dihydroxypropan-2-yl)oxy)propan-2-yl)acetamide (8)

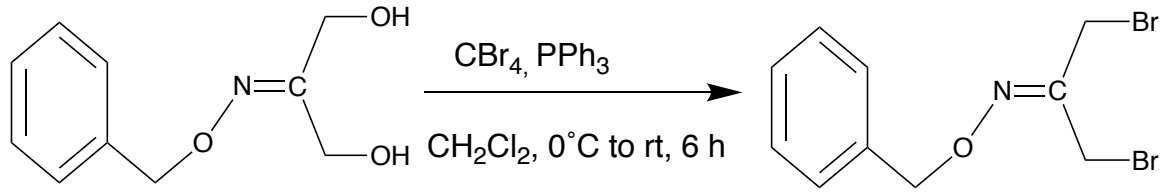


7 8

To a stirred solution of **7** (60 mg, 0.15 mmol) was dissolved in methanol (2 mL) then formic acid (18 µL, 0.45 mmol) added dropwise and Pd/C (10 %) (32.13 mg, 0.03 mmol) added very slowly in this solution. The reaction mixture was stirred for 5 h at room temperature. The resulting crude mixture was filtrated using celite, and the collected solvent was evaporated under reduced pressure to give the corresponding compound **8** (32.1 mg, 0.10 mmol, 66% yield) as a whitish sticky oil.

FT-IR (neat) 3355, 2937, 2881, 2134, 1648, 1544, 1457,1344, 1060, 906 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ4.22–4.17 (quint, 1H), 3.99 (s, 2H), 3.80–3.77 (quint, 2H), 3.72–3.62 (m, 6H), 3.60–3.55 (dd, J = 7.5, 6.0 Hz, 4H), 3.43 (quint, J = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ168.88 (C, C=O), 81.74 (CH × 2), 68.35 (CH_2 × 2), 61.24 (CH_2), 61.11 (CH_2 × 2), 61.02 (CH_2 × 2), 49.27 (CH); HRMS (ESI-TOF) *m/z* calcd for $C_{11}H_{25}N_2O_8$ $[M + H]^+$ 313.1611, found 313.1606.

1,3-dibromopropan-2-one O-benzyl oxime (10)

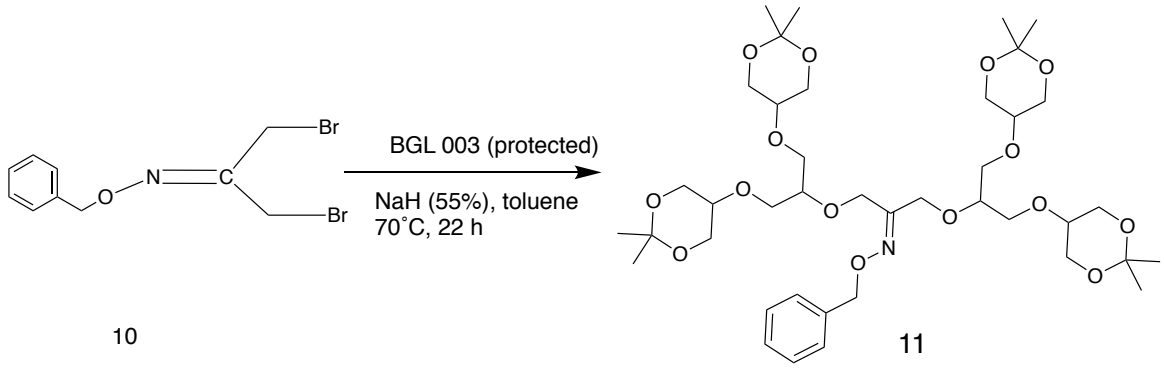


9 10

To a stirred solution of **9** [22] (976.1 mg, 5.0 mmol) in dichloromethane (15 mL) was added CBr_4 (3.48 gm, 10.50 mmol) and PPh_3 (2.69 gm, 10.25 mmol) and the reaction mixture was stirred for 6 h from 0˚C to room temperature. The resulting mixture was filtered, and the filtrate was concentrated in *vacuo*. The residue was purified by column chromatography on silica gel eluted by dichloromethane/hexane (1/2) to afford the corresponding compound **10** (1.27 gm, 3.96 mmol, 79%) as a colorless sticky oil.

FT-IR (neat) 3032, 2936, 2882, 1607, 1497, 1454, 1426, 1367, 1206, 1141, 1081, 1017, 911, 846, 828, 736, 698, 627, 569 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ7.36–7.33 (m, 5H, aromatic), 5.19 (s, 2H, Ph–C**H**$_2$–O), 4.18 (s, 2H, C–C**H**$_2$–Br) and 4.15 (s, 2H, C–C**H**$_2$–Br); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 151.57 (C, C=N), 136.80 (C, aromatic), 128.48 (CH × 2, aromatic), 128.16 (CH, aromatic), 128.05 (CH × 2, aromatic), 77.01 (CH_2), 29.56 (CH_2) and 18.50 (CH_2); HRMS (ESI-TOF) m/z calcd for $C_{10}H_{11}NOBr_2Na$ $[M+Na]^+$ 341.9105, found 341.9121.

[1,3-bis((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)oxy)propan-2-one O-benzyl oxime] (11)

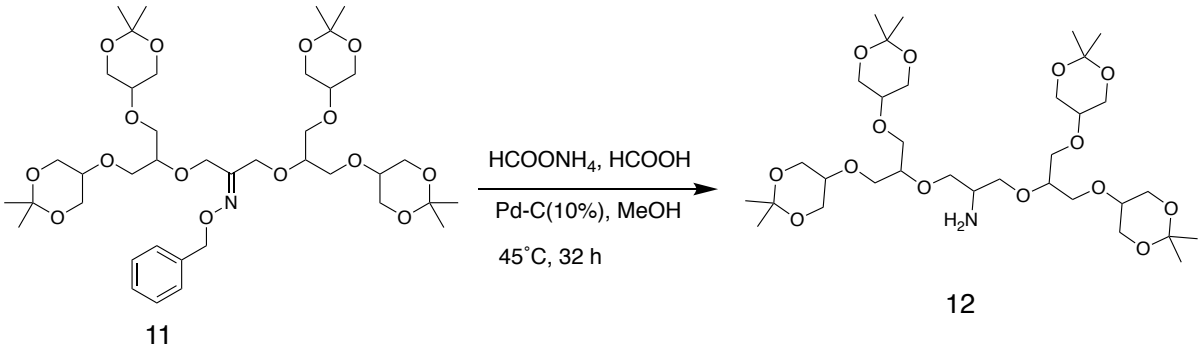


10 11

To a stirred solution of **10** (3.69 gm, 11.50 mmol) in toluene (23 mL) was added NaH (55%) (1.49 gm, 34.50 mmol) and BGL003(protected) (8.10 gm, 25.29 mmol), and the mixture was stirred for 22 h at 70˚C. The resulting reaction mixture was concentrated in vacuo, and then brine (30 mL) was poured, extracted with ethyl acetate (100 mL × 3). The resulting reaction mixture was concentrated in *vacuo*. The combined organic layers were dried over sodium sulfate (Na_2SO_4) and concentrated in *vacuo*. The crude residue was purified by silica gel column chromatography, eluted with dichloromethane/acetone (3/1) to afford the corresponding compound **11** (6.70 gm, 8.37 mmol, 72 %) as a colorless sticky oil.

FT-IR (neat) 2992, 2873, 2306, 2247, 1455, 1372, 1251, 1288, 1199, 1094, 1043, 937, 830, 733, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.30 (m, 5H), 5.09 (s, 2H), 4.47 (s, 2H), 4.25 (s, 2H), 3.95–3.90 (m, 8H), 3.72–3.67 (m, 8H), 3.64–3.60 (m, 2H), 3.58–3.50 (m, 8H), 3.44–3.39 (m, 4H), 1.42, 1.41 and 1.39 (s, 24H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.04 (C, C=N), 137.5 (C, aromatic), 128.4 (CH × 2, aromatic), 128.2 (CH × 2, aromatic), 128.0 (CH, aromatic), 98.2 (C × 4), 78.6 (CH), 77.3 (CH), 76.3 (CH_2), 71.01 (CH × 2), 70.98 (CH × 2), 68.4 (CH_2), 68.3 (CH_2), 67.7 (CH_2 × 2), 62.7 (CH_2 × 2), 62.60 (CH_2 × 2), 62.58 (CH_2 × 2), 62.55 (CH_2 × 2), 62.53 (CH_2 × 2), 24.7 (CH_3 × 2), 24.5 (CH_3 × 2), 22.8 (CH_3 × 2) and 22.6 (CH_3 × 2); HRMS (ESI-TOF) m/z calcd for $C_{40}H_{65}NO_{15}Na$ $[M+Na]^+$ 822.4252, found 822.4260.

[1,3-bis((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)oxy)propan-2-amine] (12)



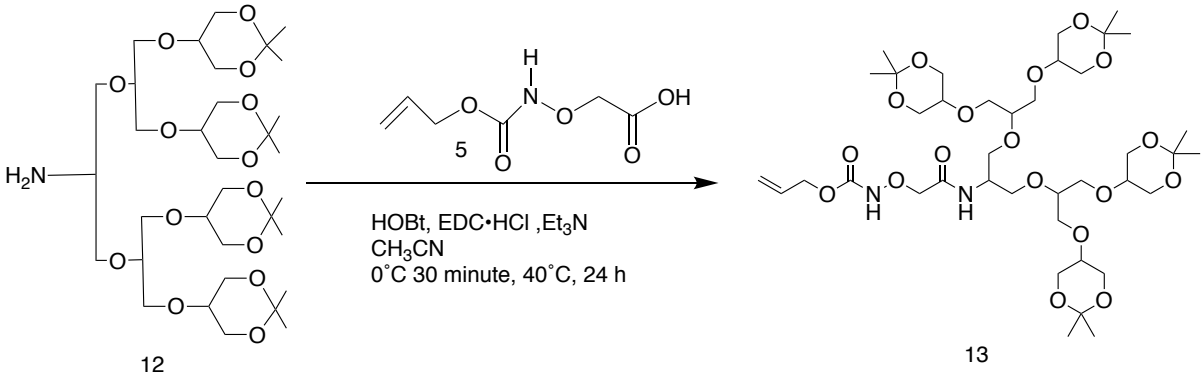
11 12

A stirred solution of **11** (1.70 gm, 2.12 mmol) was dissolved in methanol (45 mL). A mixture of ammonium formate (0.67 gm, 10.62 mmol) and formic acid (641 µL, 17.01 mmol) was added drop by drop into the solution after 30 minutes. The reaction was cooled at 0˚C for about 30 minutes, and Pd/C (10%) (0.45 gm, 0.42 mmol) was added portion by portion very slowly into that solution, and the reaction mixture was stirred for 32 h at 45˚C. The resulting mixture was filtered, washed with brine (20 mL × 2), poured into saturated sodium bicarbonate (20 mL × 2), extracted with dichloromethane (100 mL × 3). The combined organic layers were dried over potassium carbonate and concentrated in *vacuo*. The residue was purified by silica gel column chromatography, eluted with dichloromethane/acetone (4/1) as an eluent to afford the corresponding compound **12** (0.95 gm, 1.36 mmol, 61%) as a colorless sticky oil.

FT-IR (neat) 2992, 2873, 2306, 2247, 1455, 1372, 1251, 1288, 1199, 1094, 1043, 937, 830, 733, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.97 (dd, J = 6.0, 2.5 Hz, 8H), 3.76–3.72 (m, 8H), 3.63–3.54 (m, 12H), 3.48–3.43 (m, 6H), 3.15–3.10 (m, 1H), 1.44 (s, 12H) and 1.41 (s,

12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 98.20 (C × 4), 78.56 (CH), 72.81 (CH), 71.05 (CH × 4), 68.63 (CH_2), 68.56 (CH_2), 62.59 (CH_2 × 4), 62.52 (CH_2 × 8), 51.30 (CH) 24.41 (CH_3 × 4) and 22.79 (CH_3 × 4); HRMS (ESI-TOF) m/z calcd for $C_{33}H_{61}NO_{14}Na$ $[M+Na]^+$ 718.3390, found 718.4010.

allyl(2-((1,3-bis((1,3-bis((2,2-dimethyl-1,3-dioxan-5-yl)oxy)propan-2-yl)oxy)propan-2-yl)amino)-2-oxoethoxy)carbamate (13)

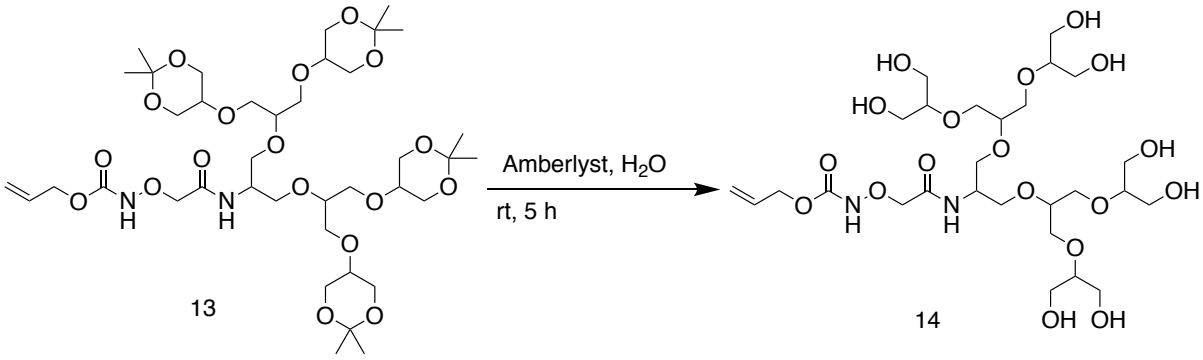


To a stirred solution of 2-((((allyloxy)carbonyl)amino)oxy)acetic acid **5** [21] (125 mg, 0.71 mmol) in acetonitrile (3 mL) was cooled at 0˚C. The reaction mixture was stirred at 30 minutes, and HOBt (143.17 mg, 0.93 mmol), EDC•HCl (170 mg, 0.93 mmol) was added to this solution, and the suspension was stirred for about 30 minutes. The reaction mixture was gradually warm to room temperature, and the compound **12** (500 mg, 0.71 mmol) triethylamine (300 µL, 2.15 mmol) was added to this reaction. The reaction mixture was stirred at 40˚C at 24 h. The resulting crude mixture was evaporated, extracted with ethyl acetate (60 mL × 3), dried over sodium sulfate (Na_2SO_4). After filtration, the solvent was concentrated in *vacuo*. The reaction crude mixture was purified by silica gel chromatography using dichloromethane/methanol (7/1) to afford the corresponding compound **13** (400 mg, 0.46 mmol, 65%) as a yellowish sticky oil.

FT-IR (neat) 3510, 3310, 2991, 2939, 2874, 1739, 1672, 1535, 1455, 1373, 1334, 1228, 1199, 1096, 1043, 939, 829, 755, 732, 666 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97–5.89 (m, 1H), 5.35 (dd, J_{trans}= 17.0, 1.0 Hz, 1H), 5.28 (dd, J_{cis} = 10.5, 1.0 Hz, 1H), 4.66 (d, J = 5.5 Hz, 2H), 4.41 (s, 2H), 4.25 (quint, J = 5.0 Hz, 1H), 4.0–3.96 (m, 8H), 3.79–3.69 (m, 12H), 3.66–3.54 (m, 10H), 3.46–3.42 (m, 4H), 1.44 (d, J = 5.0 Hz, 12H) and 1.42 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.46 (C, C=O), 157.67 (C, C=O), 131.80 (CH), 118.72 (CH_2), 98.28 (C × 4), 78.72 (CH), 77.23 (CH), 75.39 (CH_2), 71.19 (CH × 2), 71.07 (CH × 2), 68.85 (CH_2 × 2), 68.72 (CH_2

× 2), 68.60 (CH_2 × 2), 66.54 (CH_2), 62.53 (CH_2 × 2), 62.49 (CH_2 × 2), 62.44 (CH_2 × 2), 62.39 (CH_2 × 2), 49.18 (CH), 23.82 (CH_3 × 2), 23.62 (CH_3 × 2), 23.56 (CH_3 × 2) and 23.40 (CH_3 × 2); HRMS (ESI-TOF) m/z calcd for $C_{39}H_{68}N_2O_{18}Na$ $[M + Na]^+$ 875.4365, found 875.4354.

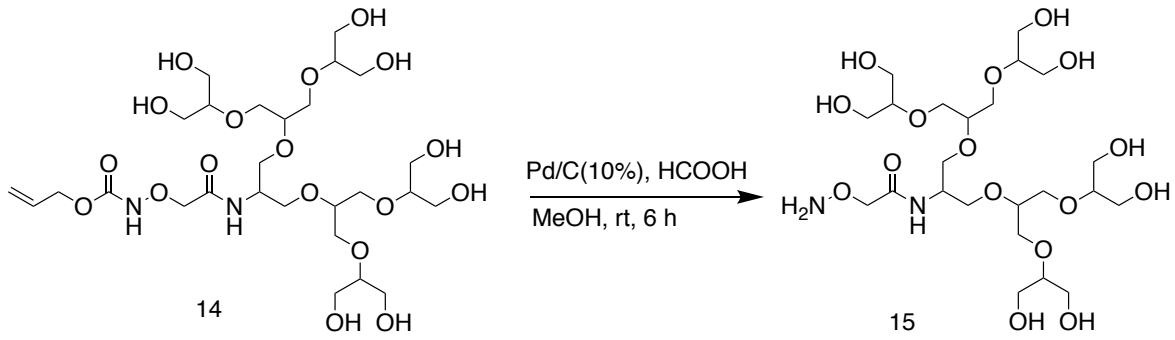
allyl (2-((5,11-bis(((1,3-dihydroxypropan-2-yl)oxy)methyl)-1,15-dihydroxy-2,14-bis(hydroxymethyl)-3,6,10,13-tetraoxapentadecan-8-yl)amino)-2-oxoethoxy)carbamate (14)



A stirred solution of **13** (140 mg, 0.16 mmol) was dissolved in H_2O (2mL), and Amberlyst®15 (140 mg) was added to this solution. The reaction mixture was stirred at room temperature for 5 h. The resulting mixture was filtered, and the solvent was concentrated in *vacuo* to afford the corresponding compound **14** (80 mg, 0.11 mmol, 70%) as a yellowish sticky oil.

FT-IR (neat) 3370, 2933, 2881, 1731, 1658, 1549, 1465, 1413, 1346, 1262, 1119, 1052 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.02–5.93 (m, 1H), 5.38–5.24 (dd, J_{trans} = 21.5 Hz, 2.0 Hz, J_{cis} = 13.0 Hz, 2H), 4.66 (d, J = 5.0 Hz, 2H), 4.34 (s, 2H), 4.24 (quint, J = 6.5 Hz, 1H), 3.79–3.71 (m, 14H), 3.68–3.58 (m, 16H) and 3.45 (quint, J = 6.0 Hz, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 169.76 (C, C=O), 158.49 (C, C=O), 132.19 (CH), 117.16 (CH_2), 81.73 (CH × 2), 81.70 (CH × 2), 78.9 (CH × 2), 75.03 (CH_2), 69.42 (CH_2 × 4), 68.50 (CH_2 × 2), 65.99 (CH_2), 61.12 (CH_2 × 4), 61.08 (CH_2 × 4) and 49.54 (CH);HRMS (ESI-TOF) m/z calcd for $C_{27}H_{53}N_2O_{18}$ $[M + H]^+$ 693.3293, found 693.3285.

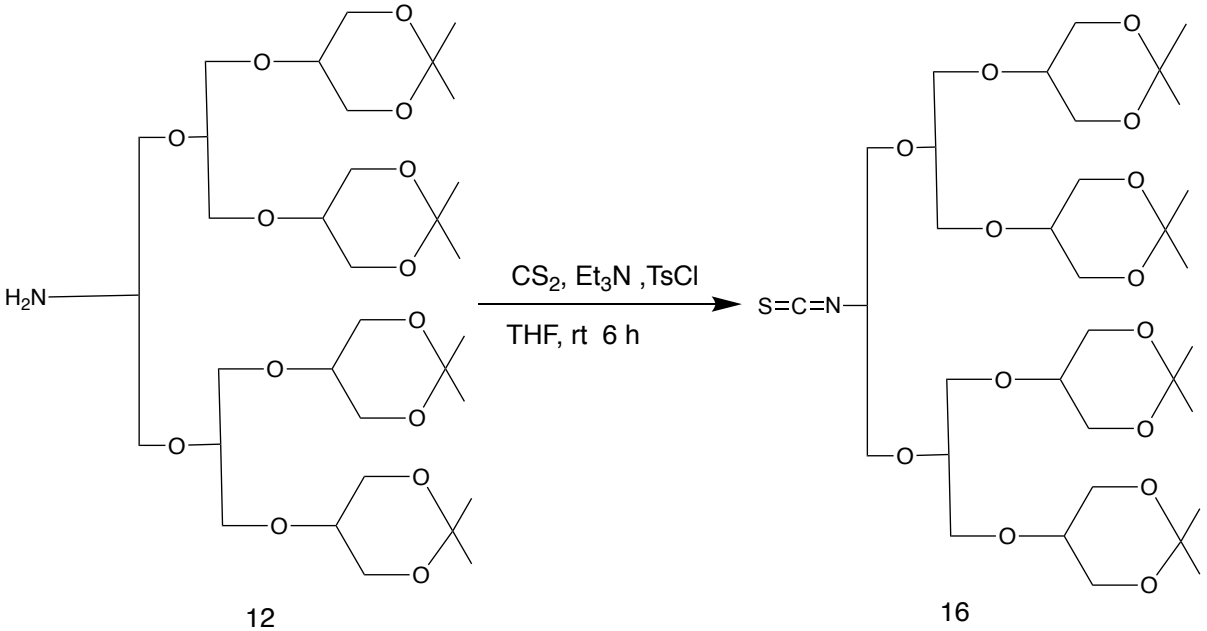
2-(aminooxy)-N-(5,11-bis(((1,3-dihydroxypropan-2-yl)oxy)methyl)-1,15-dihydroxy-2,14-bis(hydroxymethyl)-3,6,10,13-tetraoxapentadecan-8-yl)acetamide (15)



14 15

To a stirred solution of **14** (80 mg, 0.11 mmol) was dissolved in methanol (2 mL), formic acid (13 μL, 0.34 mmol) added dropwise, and Pd/C (10 %) (20 mg, 0.02 mmol) added very slowly in this solution. The reaction mixture was stirred for 6 h at room temperature. The resulting crude mixture was filtered using celite, and the collected solvent was concentrated in *vacuo* to afford the corresponding compound **15** (60 mg, 0.09 mmol, 85%) as a yellowish sticky oil. FT-IR (neat) 3355, 2937, 2881, 2134, 1648, 1544, 1457, 1344, 1060, and 906 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 4.22 (quint, J = 5.5 Hz, 1H), 4.01 (s, 2H), 3.82–3.71 (m, 13 H) 3.69–3.65 (m, 9H), 3.62–3.59 (m, 8H) and 3.45 (quint, J = 4.5 Hz, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.57 (C, C=O), 81.73 (CH × 4), 78.97 (CH × 2), 69.47 (CH_2 × 4), 68.46 (CH_2 × 2), 61.30 (CH_2), 61.13 (CH_2 × 6), 61.09 (CH_2 × 2) and 49.13 (CH); HRMS (ESI-TOF) m/z calcd for $C_{23}H_{48}N_2O_{16}Na$ $[M + Na]^+$ 631.2902 found 631.2902.

[5,5',5'',5'''-((((2-isothiocyanatopropane-1,3-diyl)bis(oxy))bis(propane-2,1,3-triyl))tetrakis(oxy))tetrakis(2,2-dimethyl-1,3-dioxane)] (16)

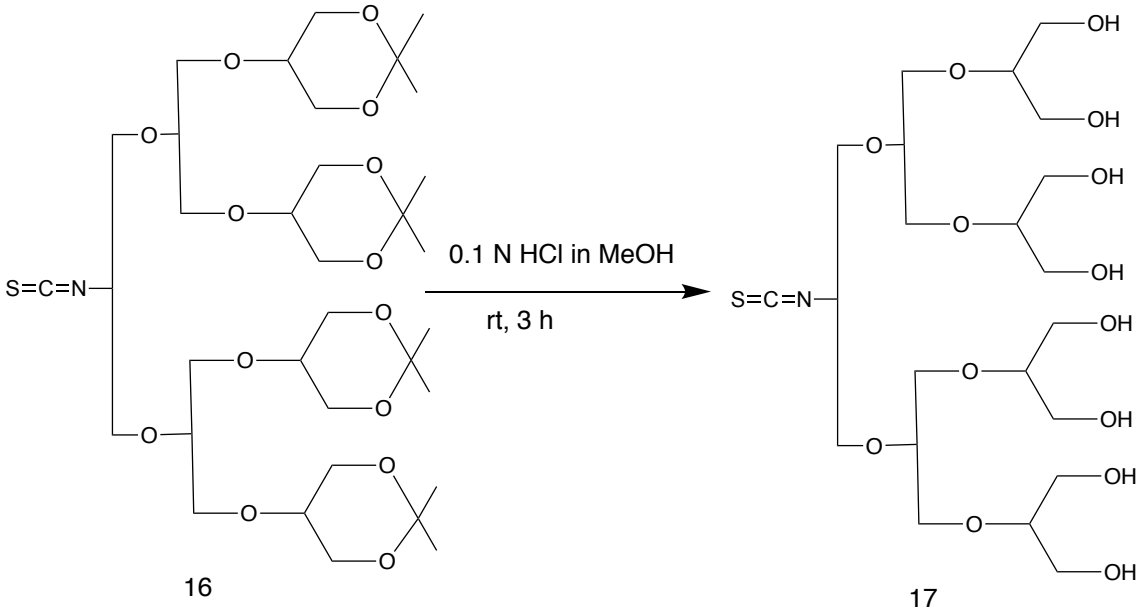


12 16

To a stirred solution of **12** (100 mg, 0.14 mmol) in THF (30 µL) was added triethylamine (140 µL, 1.01 mmol) and carbon disulfide (CS_2) (43 µL, 0.71 mmol) at 0˚C. The reaction mixture was stirred for 4 h at room temperature. The mixture was allowed to cool at 0˚C, and tosyl chloride (TsCl) (32 mg, 0.17 mmol) was added. The mixture was gradually warm to room temperature, and stirring was continued further for 2 h. The resulting reaction mixture was poured into 10% $KHSO_4$ (50 mL) very slowly and extracted with dichloromethane (60 mL × 3). The combined organic layer was washed with brine (10 mL) and dried over sodium sulfate (Na_2SO_4). After filtration, the solvent was evaporated under reduced pressure, and the reaction crude was purified by silica gel chromatography using dichloromethane/acetone (4/1) as an eluent to afford the corresponding compound **16** (90 mg, 0.12 mmol, 84%) as a colorless sticky oil.

FT-IR (neat) 2993, 2940, 2874, 2071, 1456, 1373, 1251, 1228, 1199, 1044, 938, 829, 755, 668 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.99–3.91 (m, 9H), 3.80 (dd, J = 10.0, 4.5 Hz, 2H), 3.77–3.70 (m, 10H), 3.66–3.57 (m, 6H), 3.55 (dd, J = 10.0, 5.5 Hz, 4H), 3.47–3.42 (m, 4H), 1.43 (s, 12H) and 1.40 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.68 (C, SCN), 98.12 (C × 4), 79.18 (CH), 77.39 (CH), 77.13 (CH), 76.88 (CH), 71.11 (CH × 2), 69.96 (CH_2), 68.92 (CH_2 × 4), 62.43 (CH_2 × 8), 62.39 (CH_2), 58.14 (CH), 23.89 (CH_3 × 2), 23.86 (CH_3 × 2), 23.33 (CH_3 × 2) and 23.30 (CH_3 × 2); HRMS (ESI-TOF) m/z calcd for $C_{34}H_{59}NNaO_{14}S$ $[M + Na]^+$ 760.3554, found 760.3563.

2,2'-((2-(3-((1,3-bis((1,3-dihydroxypropan-2-yl)oxy)propan-2-yl)oxy)-2-isothiocyanatopropoxy)propane-1,3diyl)bis(oxy))bis(propane-1,3-diol) (17)



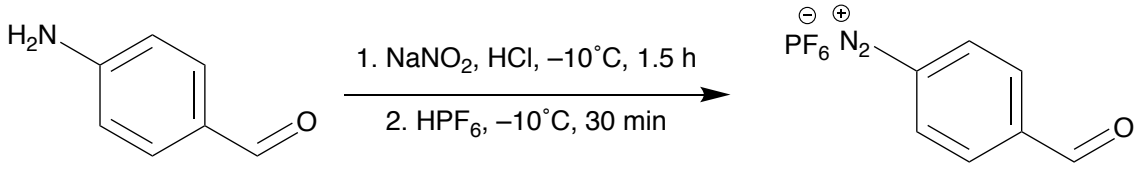
16 17

A stirred solution of **16** (1.60 gm, 2.18 mmol) was dissolved in 10 mL (0.1N HCl in MeOH) hydrochloride solution and stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure to afford the corresponding compound **17** (1.2 gm, 2.08 mmol, 96%) colorless sticky oil.

FT-IR (neat) 3372, 2933, 2876, 2106, 1653, 1541, 1458, 1401, 1345, 1263, 1204, 1119, 1071, 831, 677 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 4.07 (quint, J = 4.8 Hz, 1H), 3.89–3.55 (m, 30H) and 3.43 (quint, J = 4.4 Hz, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 135.42 (C, SCN), 83.12 (CH × 2), 83.10 (CH × 2), 80.6 (CH × 2), 71.01 (CH_2 × 3), 70.77 (CH_2 × 3), 62.53 (CH_2 × 4), 62.52 (CH_2 × 4) and 59.51 (CH); HRMS (ESI-TOF) m/z calcd for $C_{22}H_{43}NNaO_{14}S$ $[M + Na]^+$ 600.2302, found 600.2306.

EXPERIMENTAL FOR CHAPTER 3

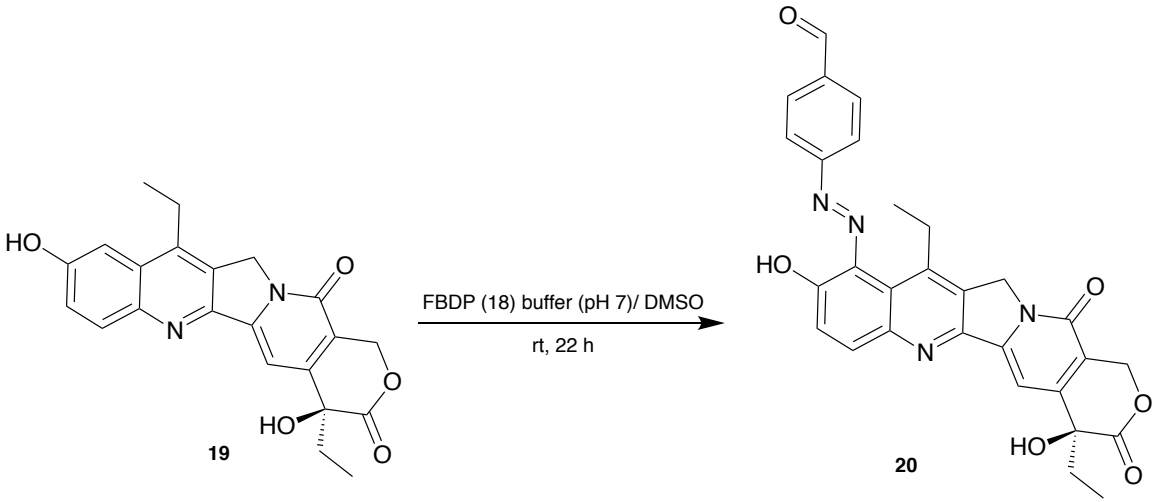
***Procedure for the synthesis of compound* 18** [32]



FBDP **(18)**

To a stirred suspension of 4-amino benzaldehyde polymer (5.00 g, 41.3 mmol) in 12N HCl (85 mL) was added the solution of sodium nitrite (3.42 g, 49.5 mmol) in water (67 mL) at –10˚C. The resulting solution was stirred at –10˚C. After 1.5 h, 60% hexafluorophosphoric acid in water (10.3 mL, 70.2 mmol) was added at –10˚C and stirred for 30 min. Then the reaction mixture was further stirred at room temperature for 30 min. The resulting solids were collected by filtration and washed with water and ethyl acetate, dried in *vacuo* to afford 4-Formylbenzene diazonium hexafluorophosphate (FBDP) **18** [32] (4.14 g, 36%) as an off-white solid.

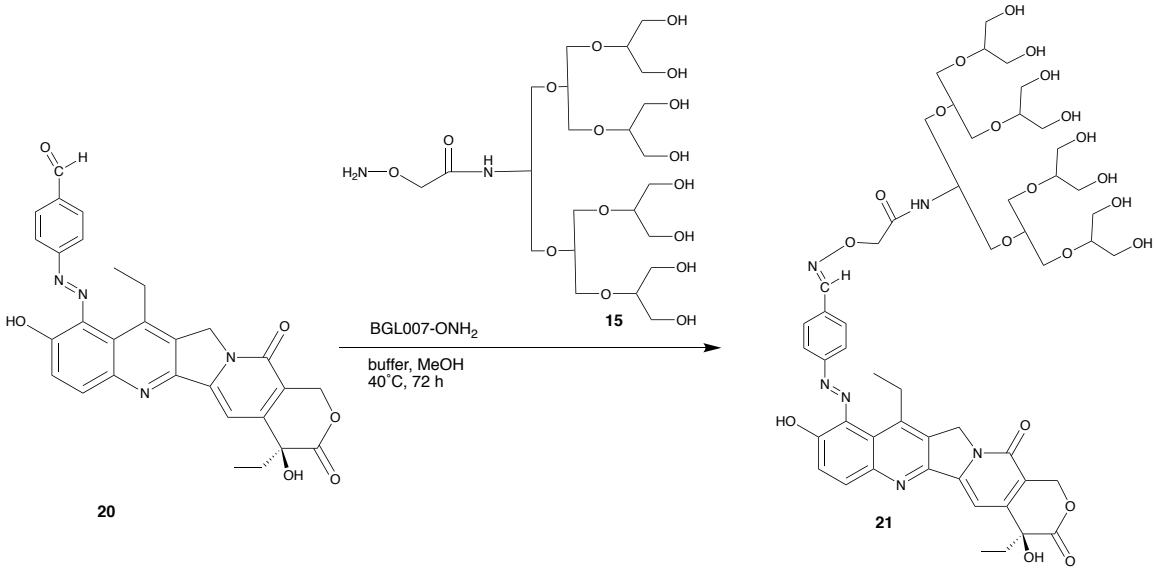
(*S*,*E*)-4-((4,11-diethyl-4,9-dihydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-pyrano[3',4':6,7]indolizino[1,2-*b*]quinolin-10-yl)diazenyl)benzaldehyde (20) [33]



To a stirred solution of SN-38 **19** (100 mg, 0.25 mmol) in 100 mM pH 7.0 NaH_2PO_4/Na_2HPO_4 buffer (8.50 mL), DMSO (4.25 mL) was added FBDP **(18)** (106 mg, 0.382 mmol) at room temperature. The resulting mixture was stirred at room temperature for 22 h, and water (15 mL) was added to the reaction mixture. The generated solid was filtered and washed with water (5 mL) and ethyl acetate (10 mL) dried in *vacuo* to afford **20** (132 mg, 95%) as a red solid.
FT-IR (KBr) 3437, 2973, 2935, 2876, 2735, 1747, 1692, 1655, 1599, 1489, 1456, 1407, 1341, 1301, 1213, 1153, 1043, 1009, 941, 834, 809, 690, 647, 617, 494 cm^{-1}; ^1H NMR (DMSO-d6,

500 MHz) δ 9.90 (s, 1H), 7.95 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 9.5 Hz, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.15 (s, 1H), 6.94 (d, J = 10 Hz, 1H), 6.53 (s, 1H), 5.37 (d, J = 5.5 Hz, 2H), 5.17 (s, 2H), 3.30 (quint, J = 7.5 Hz, 2H), 1.92–1.83 (m, 2H), 1.30 (t, J = 7.5 Hz, 3H), 0.90 (t, J = 7.5 Hz, 3H); ^{13}C NMR (DMSO-d6, 125 MHz) δ 191.9 (**C**HO), 179.5 (O**C**O), 172.9 (N**C**O), 157.0 (C), 150.5 (C), 149.7 (C), 148.2 (C), 146.8 (C), 146.1 (C), 145.5 (C), 144.3 (C), 134.1 (CH), 134.0 (CH), 132.2 (CH), 131.9 (CH), 131.9 (CH), 130.6 (C), 126.8 (C), 118.7 (CH), 117.7 (C), 99.99 (C), 96.6 (CH), 72.8 (C), 65.6 (CH_2), 50.6 (CH_2), 30.8 (CH_2), 26.7 (CH_2), 12.6 (CH_3), 8.2 (CH_3); HRMS (ESI-TOF) m/z: calcd for $C_{29}H_{24}N_4O_6Na^+$ $[M + Na]^+$, 547.1594; found, 547.1599.

N-(5,11-bis(((1,3-dihydroxypropan-2-yl)oxy)methyl)-1,15-dihydroxy-2,14-bis(hydroxymethyl)-3,6,10,13-tetraoxapentadecan-8-yl)-2-((((E)-4-((E)-((S)-4,11-diethyl-4,9-dihydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-10 yl)diazenyl)benzylidene)amino)oxy)acetamide (21)



To a stirred solution of (BGL007-ONH_2) **15** (43.45 mg, 0.07 mmol) in 100 mM pH 7.0 NaH_2PO_4/Na_2HPO_4 buffer (0.6 mL), methanol (1.78 mL) was added **20** (25 mg, 0.0476 mmol) at room temperature. The resulting solution was stirred at 40˚C for 72 h. The reaction mixture was filtered off, and the filtrate was concentrated in *vacuo*. Then the residue was washed with water (4.0 mL) and methanol (1.0 mL). The solid residue was collected, dried in vacuo to afford **21** (43.0 mg, 73%) as a deep red solid.

FT-IR (KBr) 3394, 2919, 2870, 2363, 2227, 2139, 1741, 1654, 1594, 1463, 1408, 1348, 1262, 1229, 1076, 907, 841 cm^{-1}; ^{1}H NMR (CD_3OD 500 MHz) (E:Z isomer ratio = 9:1) δ 8.14 (s, 1H), 7.95 (d, J = 9.0 Hz, 1H), 7.8 (d, J = 10.0 Hz, 1H), 7.57–7.55 (m, 1H), 7.46 (s, 1H), 7.37–7.30 (m, 2H), 6.89–6.87 (m,1H), 5.57–5.50 (m, 2H), 5.37–5.30 (m, 2H), 5.21 (s, 1H), 4.70 (s, 2H), 4.31 (quint, J = 6.5 Hz, 1H), 3.85–3.59 (m, 30H), 3.45 (quint, J = 5.0 Hz, 4H), 2.57 (quint, J = 7.5 Hz, 1H), 1.96 (d, J = 7.0 Hz, 2H), 1.31 (t, J = 7.0 Hz, 3H), 1.03 (t, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 176.5 (C, C=O), 173.37 (C, C=O), 157.6 (C, C=O), 155.07 (C), 151.14 (CH), 149.60 (C), 147.77 (C), 147.60 (C), 146.19 (C), 142.07 (C), 140.71 (C), 138.47 (C), 130.32 (CH), 129.7 (C), 128.68 (CH), 128.61 (CH), 128.59 (CH), 128.5 (CH), 128.53 (C), 118.30 (C), 117.59 (CH), 97.59 (CH), 81.75 (CH × 3), 81.73 (CH × 3), 78.9 (CH_2), 72.83 (C), 72.73 (C), 69.7 (CH_2), 69.48 (CH_2), 69.43 (CH_2), 69.32 (CH_2), 68.48 (CH_2×2), 65.32 (CH_2), 61.14 (CH_2 × 8), 51.36 (CH), 49.48 (CH_2), 30.88 (CH_2), 26.65 (CH_2), 11.69 (CH_3), 6.87 (CH_3); HRMS (ESI-TOF) m/z calcd for $C_{52}H_{70}N_6O_{21}Na$ $[M + Na]^+$ 1137.4492, found 1137.4520.

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