MULTI-FUNCTIONALIZATION OF MACROMOLECULES BY SEQUENTIAL 1,3-DIPOLAR CYCLOADDITIONS

by

PETR ANDREYEVICH LEDIN

(Under the direction of Geert-Jan Boons)

ABSTRACT

Macromolecules with two or more types of reactive groups for modification provide versatile multi-functional scaffolds, which allow precise tuning of molecular properties, tailored to a particular function. Dendrimers and polymers with multiple sites for attachment of various biologically active moieties such as carbohydrates, peptides, nucleotides and drugs have an immense potential in biomedical applications and material science. Functionalization of macromolecules with biomolecules having rich functionality requires chemoselective and efficient reactions to avoid a need for protecting groups and hence post-modification deprotection steps. A novel synthetic methodology for efficient dendrimer assembly and hetero-bi-functionalization based on three sequential azide-alkyne cycloadditions is described in chapters 2 and 3. The methodology is compatible with biologically important compounds rich in chemical functionalities such as peptides, carbohydrates and fluorescent tags. A strain-promoted azide-alkyne cycloaddition (SPAAC) between polyester dendrons modified at the focal point with an azido and 4-dibenzocyclooctynol moiety provided dendrimers bearing terminal and TMS-protected alkynes at the periphery. The terminal alkynes were outfitted with azido-modified polyethylene glycol chains or galactosyl residues using Cu(I) catalyzed azide-alkyne

cycloadditions (CuAAC). Next, a one-pot TMS-deprotection and second CuAAC reaction of the resulting terminal alkyne with azido-containing compounds gave multi-functional dendrimers bearing complex biologically active moieties at the periphery.

Nitrile oxides and azides undergo facile 1,3-dipolar cycloadditions with cyclooctynes and provide an orthogonal pair of functional groups for sequential metal-free click reactions. Such selectivity makes it possible to multi-functionalize biomolecules and materials by simple synthetic procedures that do not require toxic metal catalysts. In chapter 4 we demonstrate the utility of this methodology by developing a unique synthetic route to oxime and azide bearing block copolymers *via* reversible addition-fragmentation chain transfer copolymerization of 4-vinylbenzaldehyde and 1-(chloromethyl)-4-vinylbenzene with styrene. These block copolymers served as scaffolds for attachment of hydrophobic and hydrophilic moieties by sequential strain-promoted alkyne-azide cycloaddition and strain-promoted alkyne-nitrile oxide cycloaddition reactions.

INDEX WORDS: Dendrimer, Block copolymer, Cyclooctyne, Azide, Nitrile oxide, Multi-functionalization, CuAAC, SPAAC, SPANOC

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DEDICATION

To my family.

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TABLE OF CONTENTS

Page

APPENDICES

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SCHEMES

Page

LIST OF ABBREVIATIONS

NMR	Nuclear Magnetic Resonance
AIBN	Azobisisobutyronitrile
ATRP	Atom Transfer Radical Polymerization
AzMVB	1-(Azidomethyl)-4-vinylbenzene
BAIB	Bis(acetoxy)iodobenzene
BARAC	Biarylazacyclooctynone
bis-MPA	2,2-Bis(hydroxymethyl) propionic acid
Boc	Tert-butoxycarbonyl
BPO	Benzoyl peroxide
COSY	Correlation Spectroscopy
CT	Computer Tomography
CTA	Chain Transfer Agent
CuAAC	Copper-Catalyzed Azide-Alkyne Cycloaddition
DBN	Di-tert-butyl-N-oxide
DCC	*N, N'*-Dicyclohexyl carbodiimide
DCM	Dichloromethane
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DIBO	4-Dibenzylcyclooctynol

DIC	*N,N'*-Diisopropylcarbodiimide
DIFO	6,6-Difluorocyclooct-4-ynol
DIPEA	*N,N*-Diisopropylethylamine
DLS	Dynamic Light Scattering
DMAP	4-Dimethylaminopyridine
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOTA	1,4,7,10-Tetraazacyclododecane-1,4,7,10-Tetraaceetic acid
EDTA	Ethylenediaminetetraacetic acid
EPR effect	Enhanced Permeability and Retention
EtOAc	Ethyl acetate
Fmoc	Fluorenylmethyloxycarbonyl
FRET	Förster Resonance Energy Transfer
FT-IR	Fourier Transform Infrared Spectroscopy
GPC	Gel Permeation Chromatography
HBTU	2-(1*H*-benzotriazole-1-yl)-oxy-1,1,3,3-tertamethyl hexafluorophosphate
HIV	Human Immunodeficiency Virus
HOBt	Hydroxybenzotriazole
HPAEC	High-Performance Anion Exchange Chromatography

HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
MALDI-TOF	Matrix Assisted Laser Desorption/Ionization-Time Of Flight
M_n	Number average molecular weight
MRI	Magnetic Resonance Imaging
MTX	Methotrexate
M_w	Weight average molecular weight
MW	Molecular Weight
NHS	*N*-hydroxy succiniimide
NMM	*N*-Methylmorpholine
NMP	Nitroxide-Mediated radical Polymerization
OCT	Cyclooct-2-ynol
PAMAM	Poly(amidoamine) dendrimer
PCL	Polycaprolactone
PDI	Polydispersity index
PEG	Polyethylene glycol
PLA	Poly-D,L-lactide
PMDETA	*N,N,N',N'',N''*-Pentamethyldiethylenetriamine
PyBop	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate

RAFT	Reversible Addition-Fragmentation Chain Transfer
RGD	Arginylglycilaspartic acid
RI	Refractive Index
ROMP	Ring Opening Metathesis Polymerization
ROP	Ring Opening Polymerization
RuAAC	Ruthenium-catalyzed Azide-Alkyne Cycloaddition
SPAAC	Strain-Promoted Azide-Alkyne Cycloaddition
SPADC	Strain-Promoted Alkyne-Diazocarbonyl Cycloadditions
SPANC	Strain-Promoted Alkyne-Nitrone Cycloaddition
SPANOC	Strain-Promoted Alkyne-Nitrile Oxide Cycloaddition
TBAF	Tetra(n-butyl)ammonium fluoride
TBTA	Tris(benzyltriazolyl)methyl amine
TEM	Transmission Electron Microscopy
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin Layer Chromatography

TMS	Trimethylsilyl
UV-Vis	Ultraviolet-Visible spectroscopy
VBA	4-Vinylbenzaldehyde
VBC	1-(Chloromethyl)-4-vinylbenzene

CHAPTER 1

INTRODUCTION AND LITERATURE OVERVIEW

Robust, efficient, and orthogonal reactions in material science

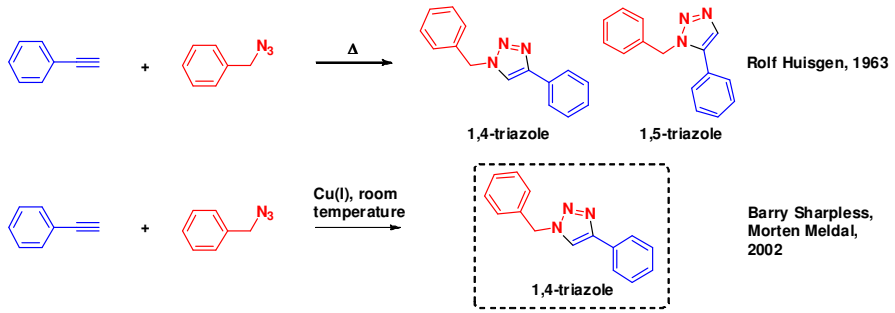
Traditionally, chemical transformations used to functionalize macromolecules would depend on a type of monomer constituting the polymer or dendrimer. For instance, the coupling between amines and carboxylic acids to give amides can be used to modify polyamide-based dendrimers or polyacrylates.[1] Conjugation of biomolecules such as nucleic acids, carbohydrates, and peptides to macromolecular scaffolds, however, demands reactions with exceptional selectivity and efficiency. The search for these reactions resulted in creation of the subclass of "click" transformations satisfying strict requirements such as: benign conditions, equimolarity of reagents, ease of purification, fast timescale, high yields, stability of linkage, and chemoselectivity.[2] The class includes variations of thiol-ene reactions, carbonyl based reactions, Diels-Alder reaction, and [3+2] cycloadditions between alkynes and azides.[3] The latter is universally accepted as one of the most versatile coupling reactions for use in material science and bioconjugation.

Copper-catalyzed azide-alkyne cycloadditions

The [3+2] cycloaddition reactions of alkynes with azides are known for more than a century. Rolph Huisgen and coworkers studied 1,3-dipolar cycloadditions thoroughly in the mid 20th century.[4] It was found that although the formation of a triazole was exothermic, the high activation barrier demanded elevated temperatures for reaction to proceed. Furthermore, the thermal reaction is not regioselective giving both 1,4 and 1,5-substituted 1,2,3-triazoles (Scheme

1.1). Activated terminal alkynes such as propiolates react with azides under milder conditions[5] but are prone to side reactions such as Michael additions and are, therefore, not suitable for applications in bioconjugation where high functional group tolerance is required. The discovery of a Cu(I) catalysis of azide-alkyne cycloaddition (CuAAC) by groups of Sharpless[6] and Meldal[7] in 2002 expanded the scope of this reaction to bioconjugation and material science.[8]

Scheme 1.1 Thermal and Cu(I) catalyzed azide-alkyne cycloaddition reactions



The use of the copper catalyst results in a dramatic acceleration of the cycloaddition rate by a factor of 10^7. Due to the stepwise reaction mechanism only 1,4-disubstituted triazoles are produced. Importantly, the reaction is not sensitive to the substitution in the azide or alkyne moieties and proceeds in a wide range of solvents including physiological medium. The CuAAC therefore became a prototypical "click" reaction due to instantaneous ligation of even complex azide- and alkyne-containing molecules in virtually any conditions.[9] One of the factors that makes CuAAC an indispensable tool for bioconjugation with polymers and dendrimers is that it is unaffected by most functional groups found in biomolecules such as peptides and carbohydrates. Although copper is the most widely used catalyst for the cycloaddition to alkynes, it was found that ruthenium also can catalyze the alkyne-azide cycloaddition to yield 1,5-disubstituted triazoles.[10] The mechanism of RuAAC is, however, quite different from CuAAC

and the reaction has not yet found a wide application. CuAAC ligation can utilize a wide variety of Cu(I) sources. For aqueous reactions *in situ* reduction of $CuSO_4$ with sodium ascorbate as a reducing agent gives the best result. It is generally regarded that Cu(II) salts do not catalyze the cycloaddition directly. However, there is a growing evidence that the active Cu(I) species can be generated by reduction of Cu(II) by solvents or as a result of oxidative side reactions such as Glaser coupling.[11]

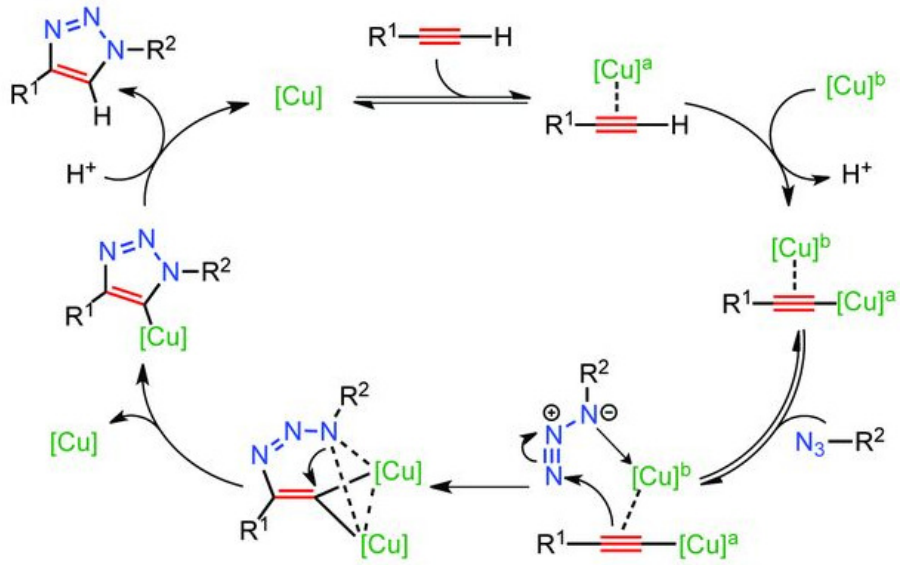


Figure 1.1 Proposed mechanism of CuAAC. Adapted from reference [12]

The most current mechanism for CuAAC[12] (Figure 1.1) involves, first, the formation of copper acetylide probably through initial π-coordination of alkyne to copper. Then the second copper atom coordinates the triple bond of the acetylide and nucleophilic N-1 of the azide. Following this step, nucleophilic attack at N-3 of the azide by the β-carbon of the acetylide forms the first covalent C–N bond. The dinuclear copper complex then undergoes ring closure by formation of the second covalent C–N bond to give copper triazolide. Formation of copper triazolide is followed by exchange of Cu^+ ion with proton from the solvent to give a triazole.

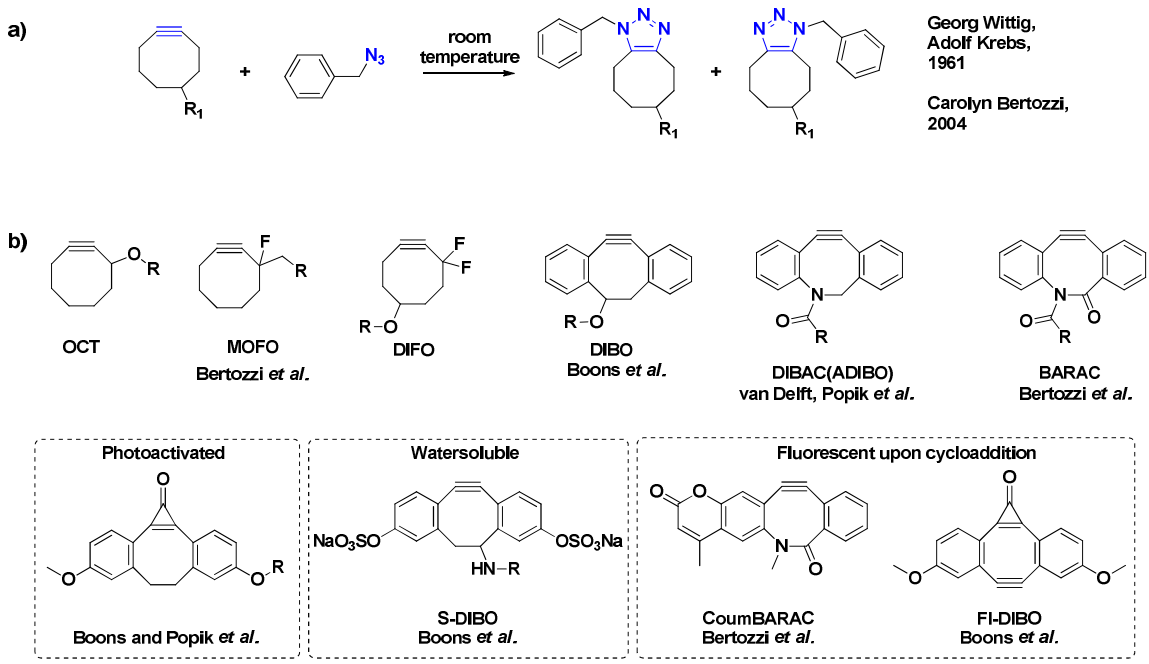
This mechanism indicates that two copper atoms participate in CuAAC reaction and signifies the role of π-coordination of alkyne which in turn supports the earlier observations of cycloadditions on α-acetylides such as iodoalkynes.[13] It was found that various ligands such as tris(benzyltriazolyl)methyl amine (TBTA) can stabilize Cu(I) species under physiological conditions and also significantly accelerate the reaction making it suitable for *in vitro* and *in vivo* labeling of biomolecules.[14] The inherent toxicity of Cu(I), however,[15] stimulated a search for analogous coupling reactions with high selectivity but not requiring transition metal catalysis.

Strain-promoted cycloadditions of 1,3-dipoles to alkynes

Strained cyclooctynes react with azides under ambient conditions without need for catalysis (Scheme 1.2a). It was shown that the large rate enhancement for strain-promoted cycloaddition of cyclooctyne is due to the decreased distortion energies for the 1,3-dipole and the alkyne needed to achieve reaction transition state.[16] Initially discovered by Georg Wittig and Adolf Crebs[17], the strain-promoted alkyne-azide cycloadditions (SPAAC) attracted a significant attention after group of Carolyn Bertozzi demonstrated the utility of this reaction for labeling of biological molecules.[18] Initially, cyclooctynes[19] such as oxycyclooctyne (OCT) (Scheme 1.2b) were employed for these reactions; however, their use was limited due to the relatively slow rates of reaction. It has been found that significant increase in the rate of SPAAC can be accomplished by appending electron-withdrawing groups to the propargylic position of cyclooctyne. For example, difluorinated cyclooctyne (DIFO)[20] reacts with azides approximately sixty-times faster than unsubstituted cyclooctyne. Boons and coworkers reported that derivatives of 4-dibenzocyclooctynol (DIBO) rapidly react with azido-containing saccharides and amino acids and can be employed for visualizing metabolically labeled glycans of living cells.[21] The acceleration of cycloaddition rates is attributed to increased ring strain as a result of appending

sp^2 hybridized benzene rings. Comparing to other cyclooctyne derivatives, DIBO is relatively easy to synthesize, and can be quickly and efficiently derivatized using hydroxyl group for attachment of a variety of probes.[22] Importantly, the structure of DIBO allows preparation of a wide variety of analogs with higher reactivity and other useful features.[23] For example S-DIBO, unlike other dibenzocyclooctynes is water soluble.[24] CoumBarac and Fl-DIBO become fluorescent upon formation of the triazole and can be used directly for visualizing azide-labeled biomolecules.[25]

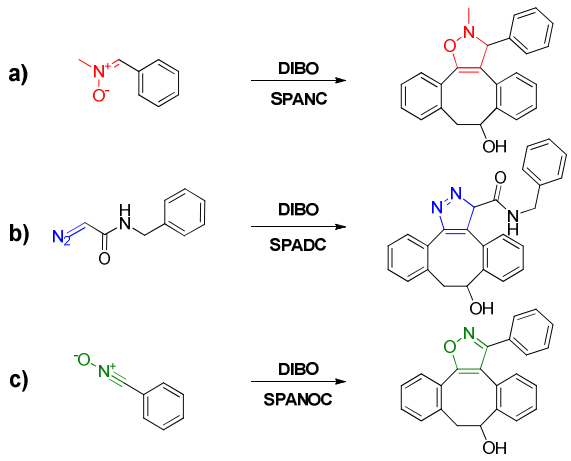
Scheme 1.2 SPAAC reaction and reagents. a) SPAAC reaction b) structures of various cyclooctynes



Due to the fact that SPAAC has high functional group tolerance, fast kinetics and does not require toxic metal catalysts, it was used for side-[26] and end-functionalization[27] of polymers, creating functional surfaces[28], polymeric networks,[29] and derivatization of dendrimers.[30] The

scope of metal free "click" reactions has further expanded when it was found that cyclooctynes could undergo fast cycloadditions with nitrones (SPANC) (Scheme 1.3).[31] The usefulness of this approach has been demonstrated by site-specific protein modification at *N*-terminal serine that could easily be converted into a nitrone and then reacted with probe-modified DIBO derivatives. In addition to azides and nitrones, nitrile oxides and diazocarbonyl derivatives readily undergo cycloadditions with DIBO to give stable pyrazoles and isoxazoles, respectively (Scheme 1.3b,c).[32] Imidoyl chlorides generate nitrile oxides and can be prepared from corresponding aldehydes. The rates of strain-promoted alkyne-nitrile oxide cycloadditions (SPANOC) were found to be fifty seven times faster than of analogous SPAAC reaction.[32]

Scheme 1.3 Strain-promoted cycloadditions of various 1,3-dipoles. a) nitrones, b) diazocarbonyls, and c) nitrile oxides with DIBO



The nitrile oxide formation can be streamlined by a direct oxidation of oximes to nitrile oxides by using a mild oxidant such as (diacetoxyiodo)benzene (BAIB).[33] Furthermore, oximes and azides provide an orthogonal pair of functional groups for sequential metal free "click"

reactions, which we exploited for construction of glycoconjugates[32] and bi-functionalization of polymers in chapter 4.

Synthesis and modification of dendrimers

Since introduction in mid 1980's[34] dendrimers are gaining an increasing popularity as a versatile multivalent platform for a host of applications. Dendrimers are emerging as promising materials for the development of imaging devices,[35] radio-therapy agents,[36] and drug and gene delivery vehicles.[37] Dendrimers were also examined as: catalysts,[38] enzyme and protein mimetic systems[39], sensors[40] and for applications in electronics.[41] Attractive properties of dendrimers include chemical homogeneity, tunability of biodistribution and pharmacokinetics by regulating size and controlled degradation by judicious choice of dendrimer chemistry.[42] Stepwise synthesis of dendrimers provides a precise control over the molecular structure and results in a very narrow molecular weight distribution comparing to the polymers. Currently, there are several synthetic approaches to dendrimers: divergent[34], convergent [43], and their combination [44] (Figure 1.2).

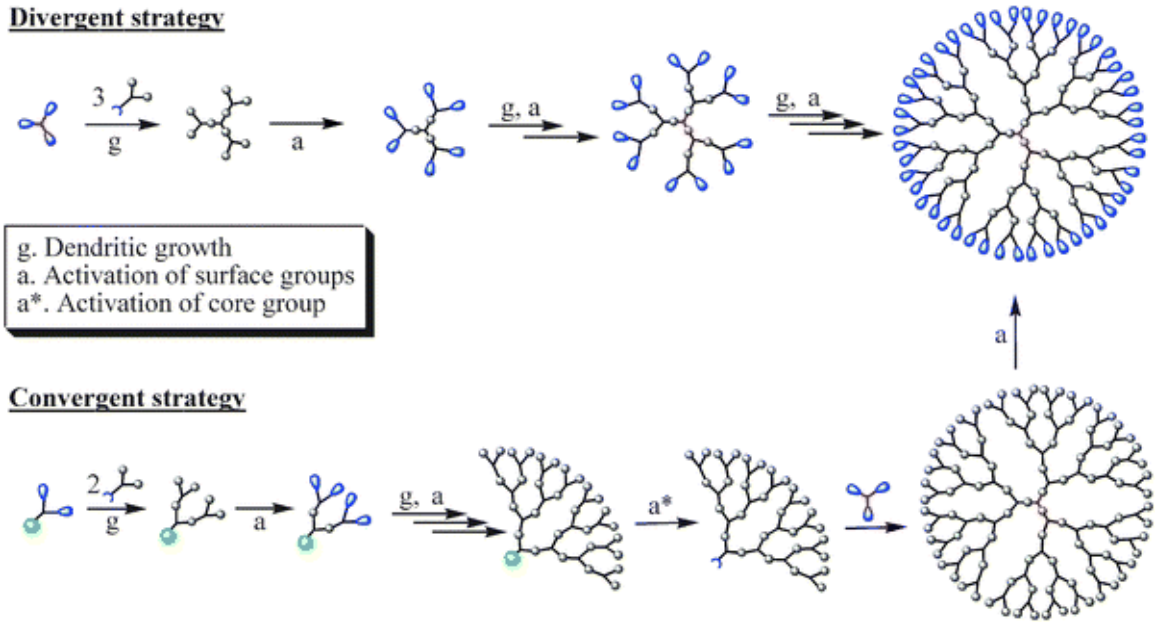


Figure 1.2 Strategies towards synthesis of dendrimers. Adapted from reference [45]

Divergent approach employs growth of the dendrimer from the core towards its periphery. Each step, when repeating units are introduced, adds one generation to the dendrimer. Therefore, when AB_2 type monomers are used, dendrimers of first generation have two surface groups, second-four, third-eight, and fourth-sixteen. Convergent synthesis starts from building blocks, which will then become periphery of the dendrimer. In the final step, dendritic wedges are joined together. Convergent approach has an advantage of relatively simple purification and precise control over the polydispersity of the dendrimer. However, steric hindrance in the dendrimer core may render the coupling reaction inefficient and lead to low yields and loss of expensive dendrons, or a necessity to use a large excess of them. Thus, the convergent assembly of dendrons into dendrimers ideally has to be quantitative and free of byproducts. Several types of dendritic backbones developed in recent years, which could be used as scaffolds for biomedical applications.

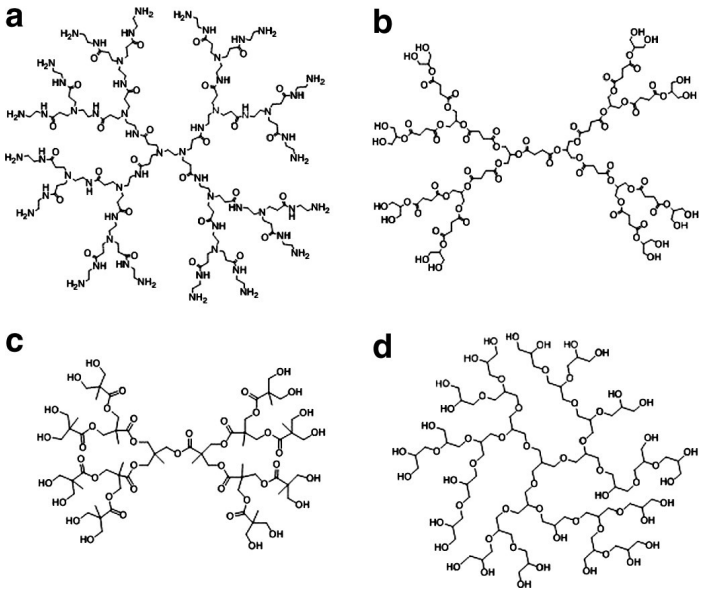


Figure 1.3 Structures of biocompatible dendrimers that have been tested for drug delivery applications. a) PAMAM, b) poly(glycerol-succinic acid) dendrimer, c) bis-MPA-based Boltorn® and, d) hyperbranched polyglycerol. Adapted from reference [46]

The driving force in finding new monomers for dendrimer synthesis is to make them water soluble, biocompatible, biodegradable, synthetically accessible, and amenable to large-scale synthesis. Commercially available polyamidoamine (PAMAM) dendrimers (Figure 1.3a), first prepared by the divergent growth approach by Tomalia *et al.* [34], are now widely used dendritic scaffolds in biology.[47] However, similarly to poly(propyleneimine) dendrimers, the presence of multiple amine groups leads to significant toxicity of unmodified amide-based dendrimers.[48] It is generally necessary to modify the surface amine groups of these dendrimers with neutral or anionic moieties to avoid toxicity and liver accumulation associated with their polycationic surfaces.[49] Polyaryl ether dendrimers developed by Fréchet and Hawker[50], on the other hand, have poor water solubility and require solubilizing groups at their periphery. Amino acid-based dendrimers, such as polylysines, are inherently biocompatible and water soluble, and are used extensively for DNA transfection and drug delivery.[51] Polyester dendrimers incorporating glycerol and succinic acid as monomers, (Figure 1.3b) have been prepared by Grinstaff *et al.*, and their potential use in tissue engineering has been demonstrated.[52] Fréchet and coworkers synthesized polyester dendrimers based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) monomer (Figure 1.3c) as candidates for the development of anticancer drug delivery systems.[53] Finally, dendritic polyethers incorporating glycerol monomers have been reported by several groups (Figure 1.3d).[54]

The typical architecture of dendrimers results in the formation of cavities, which can entrap pharmaceutically active substances.[55] Furthermore, a large number of surface functionalities – multivalency allows for tailoring of dendrimer properties: solubility, biocompatibility, and biodistribution. Surface modification of dendrimers with biomolecules benefits from high multivalent densities, which will strengthen ligand-receptor binding as a

result of a cluster effect.[56] For example, glycodendrimers[57] proved to be indispensable for studying weak carbohydrate-protein interactions. “Click” reactions are extensively used as a versatile tool for assembly and derivatization of dendrimers. Since CuAAC combines exceptional chemoselectivity with a lack of byproducts and high yields, it has been used to efficiently derivatize dendrimers with unprotected carbohydrates.[58] Wong *et al.* investigated the possibility of using oligomannose dendrons as vaccine candidates or antiviral agents. It is known that gp120 glycoprotein on the surface of the HIV virus contains a cluster of glycans with high mannose content. In attempt to mimic gp120 polyamide dendrimers having 3, 9 and 27 alkyne groups on periphery were derivatized with azido-modified tetra and nonamannosides providing a library for screening against mannose binding lectin DC-SIGN and an anti gp 120 antibody 2G12 (Figure 1.4).[58] The competition binding assays have demonstrated that the second generation dendron bearing nine Man 9 residues had at least four orders of magnitude higher affinity to 2G12 than Man 9 itself, therefore demonstrating a strong cluster glucoside effect.

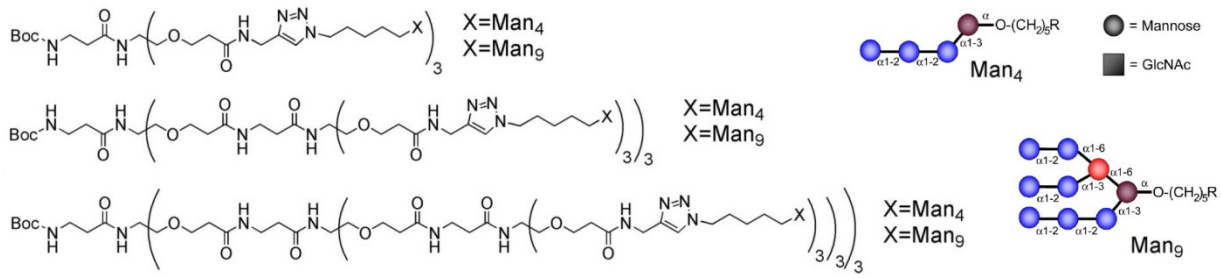
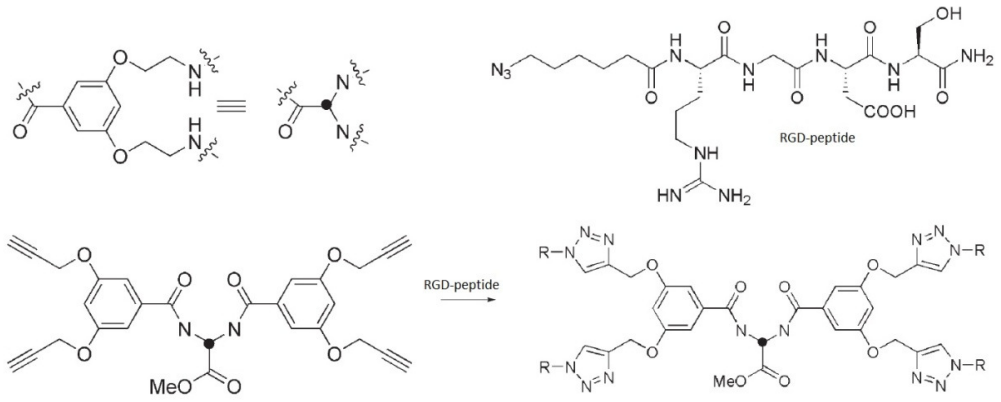


Figure 1.4 Structure of polyamide dendrimers modified with high mannose oligosaccharides *via* CuAAC. Adapted from reference [58]

This and other reports showed that glycodendrimers are a viable alternative to synthesis of complex glycans for therapeutic applications. Due to its high chemoselectivity, CuAAC also

tolerates the rich functionality of peptides and is suitable for preparation of peptide-dendrimer conjugates.[59]

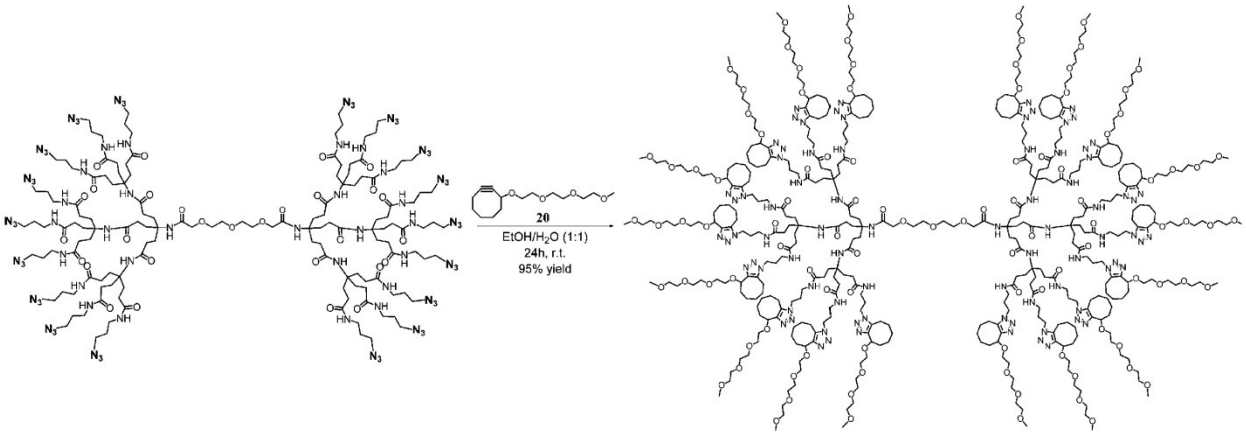
Scheme 1.4 Peripheral modification of dendrimers with RGD peptides by CuAAC. Adapted from reference [60]



For example, Liskamp *et al.* demonstrated that azido-modified peptides, including unprotected linear and cyclic arginylglycylaspartic acid (RGD) peptides, can be attached to the periphery of alkyne-bearing dendrimer. RGD peptides bind selectively to integrin proteins overexpressed on cancer cells and can be used for selective targeting of drug-delivery constructs to tumor tissue. A library of alkyne terminated dendrimers based on 3,5-dihydroxybenzoic acid as a branching unit was synthesized. First through fourth generation dendrimers were modified with various peptides utilizing $CuSO_4$/Na ascorbate catalytic system. It was found, however, that microwave heating was required to drive reactions with bulky peptides to completion.[60] One of possible reasons for low efficiency of conventional CuAAC in this case may be the scavenging of copper catalyst by coordination with peptide backbone and side chain functionality. It is also known that the residual catalyst from CuAAC coupling is difficult to remove after modification

of polyamine and polyamide-based dendrimers due to the strong chelation. Copper-free azide-alkyne cycloadditions such as SPAAC can be used to circumvent these problems.

Scheme 1.5 Peripheral modification of Newkome-type polyamide dendrimers with oligoethyleneglycol by SPAAC. Adapted from reference [30b]



SPAAC has been recently employed for surface modification of Newkome-type polyamide dendrimers having 18 azide groups on periphery by Weck *et al.* (Scheme 1.5).[30b] Initially, functionalization of a polyamide dendrimer with oligoethylene glycol was performed using CuAAC. Although the reaction was complete, all attempts of purification with column chromatography, dialysis, treatment with EDTA and precipitation gave a greenish product contaminated with ca. 0.5% of copper. The treatment of the same dendrimer with cyclooctyne-modified oligoethyleneglycol, on the other hand, gave a pure water-soluble dendrimer with minimal purification. It is important to note, however, that due to the lack of regioselectivity of SPAAC reaction, characterization of modified macromolecules with NMR becomes problematic due to presence of two isomeric triazoles. In another report, a generation 5 PAMAM dendrimer was partially derivatized with cyclooctynes (OCT) and the resulting dendritic scaffold was clicked with the azido-methotrexate (MTX) derivative.[30a] MTX is a potent antineoplastic agent

and the future research would concentrate on attachment of folic acid moieties to this dendrimer-drug conjugate to increase the cellular uptake and, therefore, overall therapeutic efficiency.

Multi-functionalization of dendrimers

Dendrimers, modified by several different peripheral entities, can combine functions of tissue targeting and imaging or be directed more precisely to a specific tissue or cell type.[61] The surface of dendrimers can be modified by prodrugs, imaging modules such as fluorescent tags, CT and MRI contrast agents,[35a] polyethylene glycol to increase water solubility and improve biocompatibility,[62] and by cell tissue targeting ligands including folic acid or RGD peptides to increase therapeutic efficiency.[63] Usually, multi-functional dendrimers are prepared by a random chemical coupling reaction, which unfortunately leads to unwanted dispersity.[64]

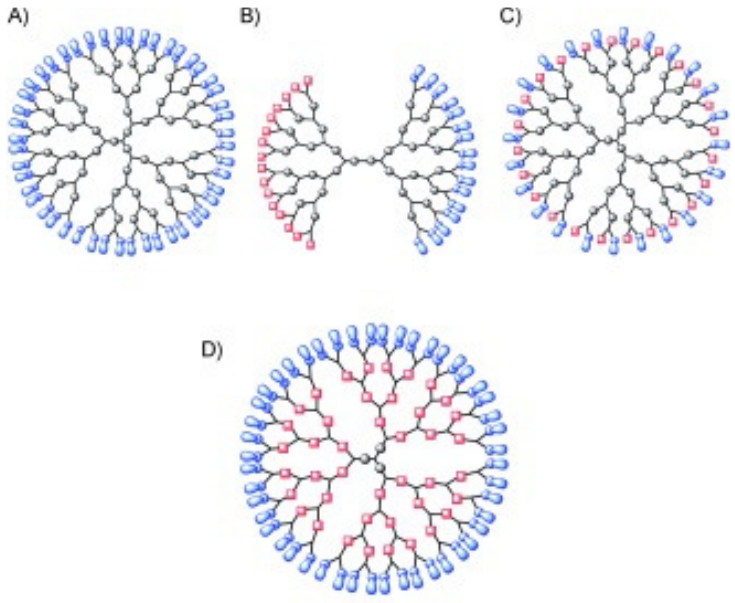
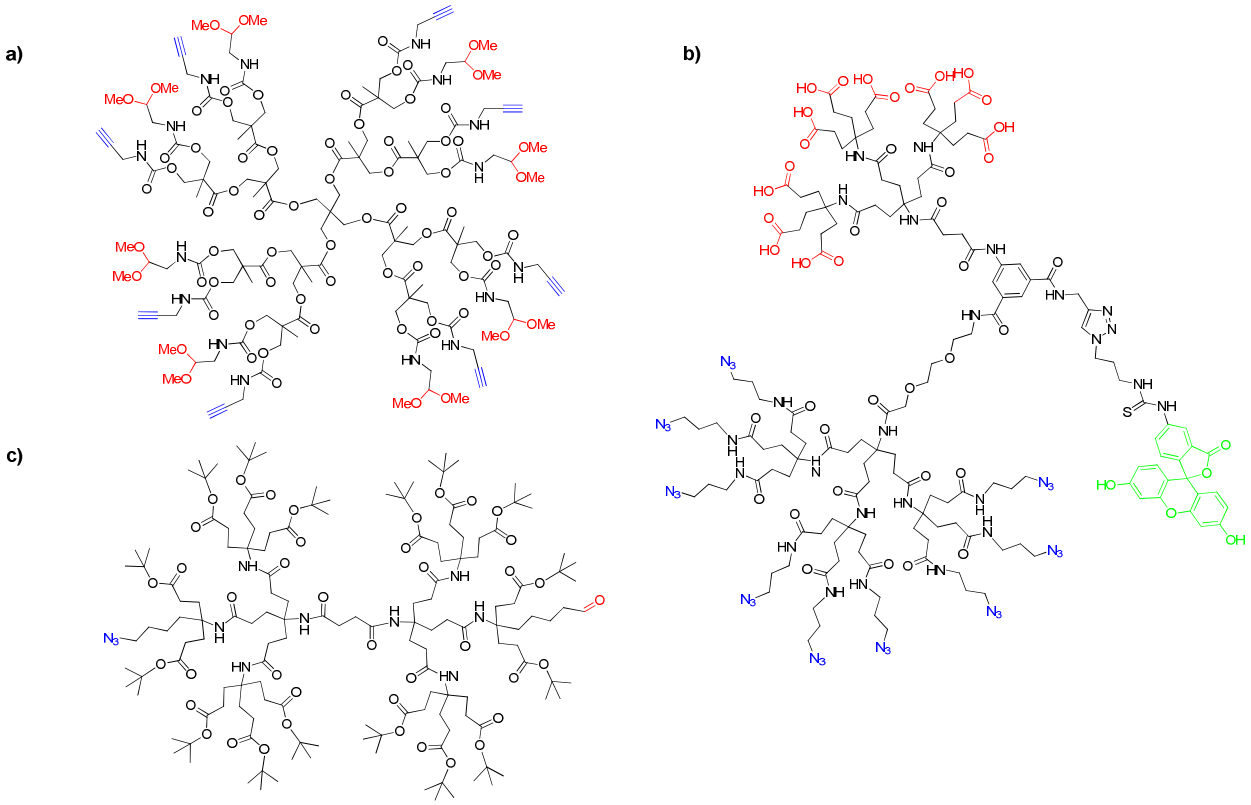


Figure 1.5 Various types of architectures of functional dendrimers. a) mono-functional dendrimer b) hetero-bi-functional Janus-type dendrimer c) peripheral bi-functional dendrimer d) bi-functional dendrimer with internal and peripheral functional groups. Adapted from reference [65]

A more attractive approach uses dendritic molecules having two or more orthogonal functionalities or protecting groups.[66] There are several ways to achieve such multi-functionality, considering convergent and divergent synthetic routes towards dendrimers. In divergent approach, mono-functional dendrimers, synthesized from AB_n monomers, have inactive interiors and active exteriors with multiple functional groups of the same type (Figure 1.5a). Two different functional groups can be present on the surface of a dendron wedge having A-type functionality, whereas the second dendron wedge has B-type functionality.[67] These Janus-type or Bow-tie dendrimers (Figure 1.5b) can be obtained by divergent, so called, double exponential growth, utilizing orthogonal protecting groups as well as a combination of divergent and convergent strategies. Wu *et al.* reported the preparation of polyester dendrimers modified by mannoside-targeting moieties and coumarin fluorescent tags by starting with a dendrimer having peripheral alcohols and isopropylidene acetals.[67a] The alcohols of the dendrimer could be modified by terminal alkynes, which could then be coupled with azide-modified coumarin. Removal of isopropylidene acetals gave alcohols, and a repetition of alkyne formation and CuAAC led to the controlled introduction of peripheral mannosides. More recently, bi-functional[68] and tri-functional[69] (Scheme 1.6b) dendrimers were constructed by "click" reaction followed by coupling of azide bearing dendron to the dendrimer core, thus enabling a surface modification by a second CuAAC. Peripheral bi-functionality of dendrimers can also be achieved by end capping of a conventional mono-functional dendrimer with ABC type monomer (Figure 1.5c). This, however, yields a strictly equal number of two functional groups on the periphery. This approach was utilized by the group of Jean Fréchet to construct the dendrimer with alkynes and acetal protected aldehyde groups on periphery (Scheme 1.6a). A bis-MPA-based dendrimer was functionalized with a 2-oxo-1,3-dioxane-5-carboxylic acid, a cyclic

carbonate, which upon treatment with corresponding amine, opens to give a carbamate and primary alcohol for subsequent derivatization.

Scheme 1.6 Examples of multi-functional dendrimers containing azide or alkyne for CuAAC and second orthogonal group enabling bi-functionalization[69-70]



To obtain the clickable bi-functional dendrimer depicted on (Scheme 1.6a) the carbonate bearing precursor was treated with 2,2-dimethoxyethanamine followed by 1,1'-carbonyldiimidazole and propargylamine.[70a] Finally, a synthetic methodology for the construction of bi-functional dendrimers with active internal and external functional groups (Figure 1.5d) can be developed based on AB_2C monomers.[65]

Various combinations of reactive groups have been installed on dendrimers for subsequent multi-functionalization. In particular, azides or alkynes for CuAAC combined with

hydroxyls for etherification[71] or aldehydes for hydrazone formation have been successfully employed as sets of the orthogonal functionalities. Weck *et al.* reported synthesis of a Newkome-type dendrimer having one azide and one aldehyde group for orthogonal bi-functionalization (Scheme 1.6c).[70b] Although the synthesis of this dendritic scaffold was cumbersome, no intermediate deprotection steps were required between treatment with biotin hydrazide to form hydrazone bond and CuAAC with propargyl glycine. Furthermore, t-butyl protecting groups can be potentially removed to reveal carboxylic acids for water solubility. Despite many attractive features of these multi-functionalization methods, the limited chemoselectivity of conventional functional groups such as alcohol, amine, carboxylic acids and carbonyls and, in some cases, the relatively large number of chemical steps for orthogonal group installation, places restrictions on the type of functionality that can be attached to a dendritic surface. Recently, protocols for sequential 1,3-dipolar cycloaddition reactions free of these disadvantages have been developed, the implementation of these novel methodologies for multi-functionalization of dendrimers will be discussed in chapters 2 and 3.

Functional polymers

The progress in nanotechnology and biomedical research is difficult without new soft materials with tunable properties and complex, yet well-defined, architectures.[72] Polymer architecture has a profound influence on physical properties of materials: crystallinity, strength, toughness, glass transition temperature (T_g) and melting temperature (T_m). The simplest arrangement of polymer chains, without branching, can be seen in linear and cyclic polymers. Branched polymers[73] have side chains attached to a main chain and include star polymers,[74] comb shaped polymers,[75] polymer brushes,[76] and hyper branched polymers (Figure 1.6b).[77] Copolymerization of different monomers adds even more diversity. Regular, random and block

copolymers, composed of two or more types of monomers, can have any of above-mentioned architectures (Figure 1.6a).[78] Historically, linear or branched polymers such as polyethylene, polyamides, and polyesters have been used mainly as commodity plastics. Past three decades have seen a tremendous progress in synthesis of bioinspired materials, which mimic proteins and nucleic acids. The structure and function of proteins and nucleic acids is dictated largely by side chain functionality, rather than a polymeric backbone.

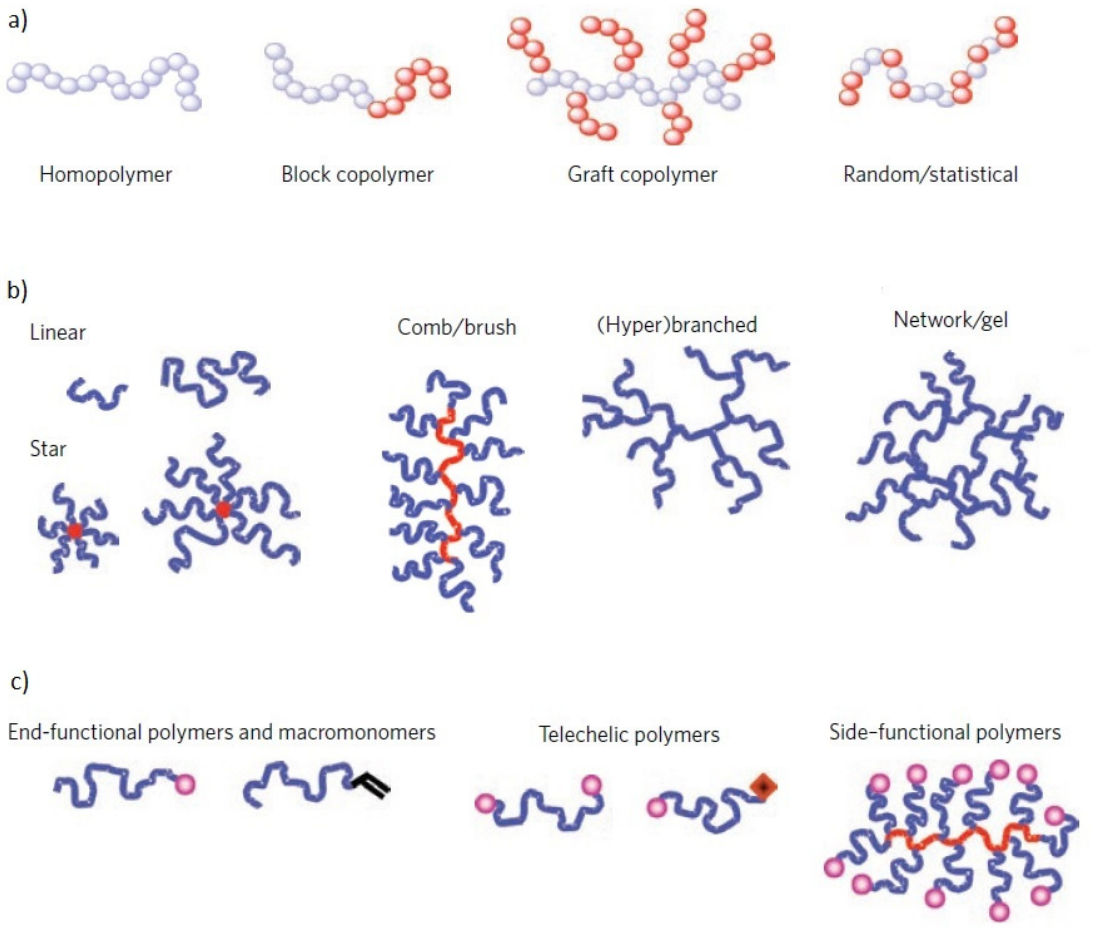


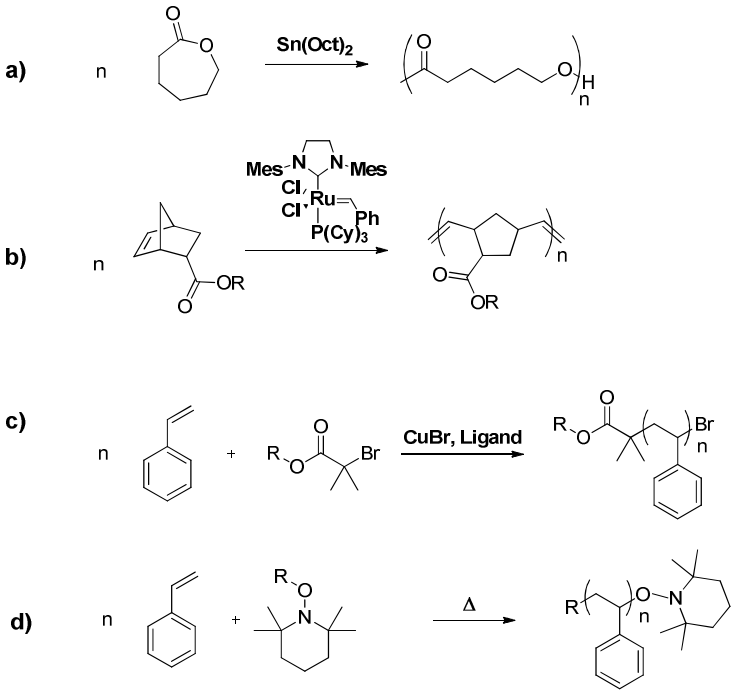
Figure 1.6 Classification of macromolecular architectures. Based on: a) composition b) topology c) functionality. Adapted from reference [79]

For example, twenty naturally occurring amino acids contain functionalities such as alcohols, amines, and carboxylic acids. Rich functionalization of proteins gives rise to intra- and

intermolecular interactions such as hydrogen bonding, Van der Waals forces, and metal complexation. These interactions determine the tertiary and quaternary structure of proteins and, hence, the biological function. Using an inspiration from nature, a new class of polymers, called functional polymers, was developed for nanotechnology and biomedical applications.[76b, 80] There are three major types of polymer functionalization: mono-end functionalization, bi-end functionalization and side chain functionalization (Figure 1.6c).

Mono-end-functional polymers contain only one functional group on one of the chain ends. These polymers were employed for synthesis of block copolymers, graft copolymers, star shaped polymers, and molecular brushes.

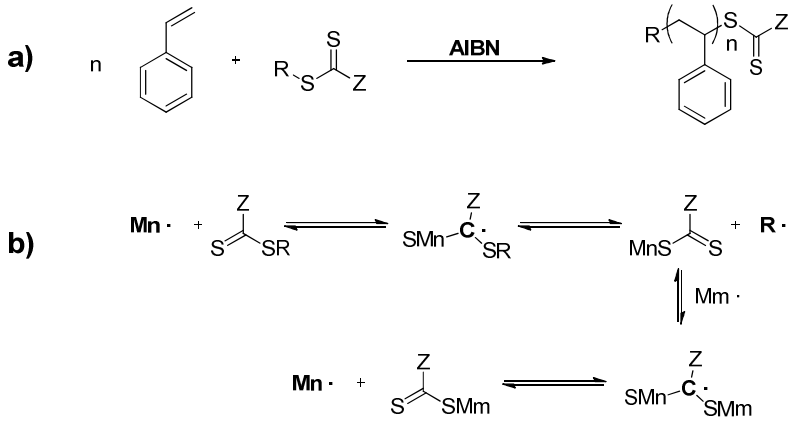
Scheme 1.7 Polymerization methods used for preparation of functional polymers. a) ROP, b) ROMP, c) ATRP, d) NMP



Usually mono-end-functionalized polymers are obtained using functional initiators or chain transfer agents equipped with chemical handles. Alternatively, such a chemical handle is

introduced by termination of polymerization with a specifically designed terminating agent. Bi-end-functional polymers bear functional groups on both ends of polymer chain. These groups can be equal or different and such polymers are called homo-bi-functional (telechelic polymers) and hetero-bi-functional polymers respectively. Several polymerization techniques yielding well-defined functional polymers with high molecular weights and low polydispersity indexes (PDI) have been developed in past three decades. Ring opening polymerization (ROP)[81] and ring opening metathesis polymerization (ROMP)[82] take advantage of a spring-loaded nature of cyclic monomers. Other polymerization techniques leading to functional polymers are controlled radical polymerization methods (CRPs). These methods include atom transfer radical polymerization (ATRP)[79, 83] and nitroxide mediated polymerization (NMP)[84] (Scheme 1.7).

Scheme 1.8 RAFT polymerization. a) general concept, b) mechanism



Reversible addition-fragmentation chain-transfer polymerization (RAFT)[78c] (Scheme 1.8) was developed in 1998 by laboratories of CSIRO in Australia[85] and is the most recent addition to free radical polymerization methods. The method employs thiocarbonylthio compounds

(dithioesters, dithiocarbamates, trithiocarbonates, and xanthates) as additives to achieve low PDI of resulting polymers. These chain transfer agents transfer the radicals between growing polymer chains, which allows for uniform molecular weight distribution as the polymer chains grow simultaneously at equal rates (Scheme 1.8b). RAFT polymerization has an advantage of high functional group tolerance. It is not moisture sensitive and can be performed at wide range of temperatures. Finally, this living polymerization can be used to prepare block copolymers and does not require the use of metal catalysts. Side chain functionalization of polymers can be achieved either by direct polymerization of monomers bearing a desired functionalization or by post-polymerization modification (Figure 1.7).[86]

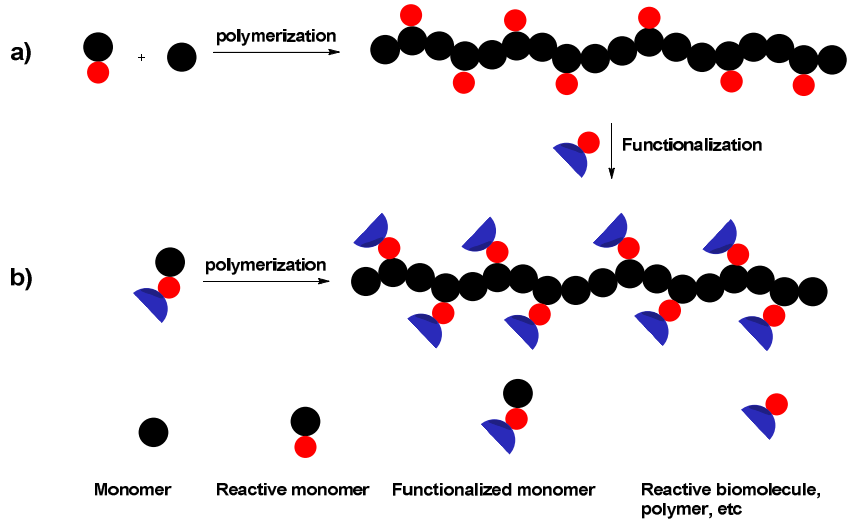
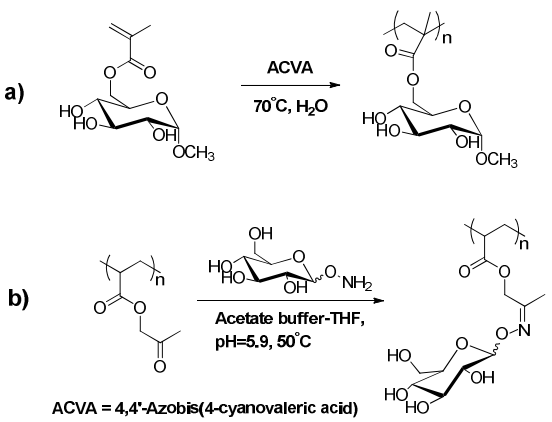


Figure 1.7 Side-chain polymer functionalization. a) grafting onto approach, b) grafting through approach

Grafting through approach uses complex pre-functionalized monomers, which after polymerization yield the target polymer without any further chemical manipulations. A number of bioinspired materials, which mimic proteins[87] and polysaccharides[88], have been synthesized from pre-functionalized monomers (Scheme 1.9a). The graft through approach is convenient

when monomer side chains are relatively non-bulky and do not contain functional groups interfering with rate and degree of polymerization. Although polymerization of complex monomers often gives satisfactory results, postpolymerization modification becomes increasingly popular due to its convenience and complexity of moieties, which can be attached to a polymer. Grafting onto polymers circumvents problems associated with complex and bulky monomers compromising the control on polymerization.[89] The graft onto approach was used to prepare complex bioconjugates such as glycopolymers[90] and peptidopolymers.[87a]

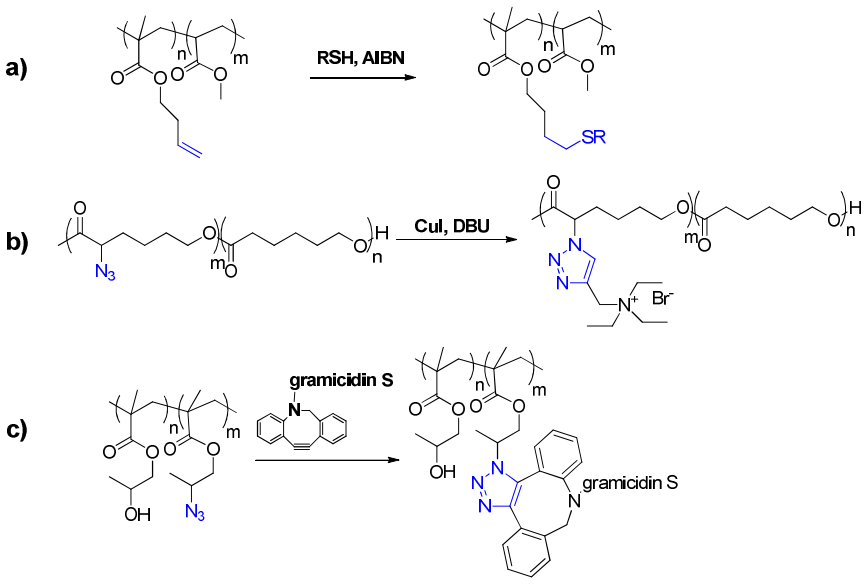
Scheme 1.9 Synthesis of glycopolymers. a) grafting through approach[88] b) graft onto approach[90]



In addition, post-polymerization modification approaches allow for modular synthesis of libraries of functional polymers from common polymeric precursors thereby offering exciting opportunities to fine tune properties of materials.[91] This method, however, requires very efficient coupling reactions between polymer chain and grafted moieties. The reason for such requirement is that functional groups on a bulky polymer chain may not be readily accessible for modification, which reduces the degree of functionalization and may introduce an unwanted heterogeneity. By far, the most widely used reaction for grafting onto polymers is CuAAC.[92]

(Scheme 1.10b). The reaction gives a stable triazole moiety as a product and both, alkyne and azide are compatible with many polymerization methods.

Scheme 1.10 Examples of "click" reactions used for grafting onto polymers. a) thiol-ene coupling[93] b) copper catalyzed azide-alkyne cycloaddition (CuAAC)[92] c) strain-promoted azide-alkyne cycloaddition (SPAAC)[26c]



CuAAC is very chemoselective and proceeds in mild conditions in a variety of solvents. For applications where the contamination with copper catalyst may interfere with the function of the polymer, metal free 1,3-dipolar cycloadditions are employed. Recently van Hest *et al.* compared the efficiency of strain-promoted and Cu-catalyzed azide-alkyne cycloadditions for grafting of antibiotic Gramicidin S peptide onto polymeric backbone (Scheme 1.10c).[26c] 2-Hydroxypropyl methacrylate was copolymerized with 2-azidopropyl methacrylate using ATRP polymerization. In this report, the efficiency of CuAAC was found to be higher than that of SPAAC, which afforded modification of only 70% of the azide groups. Authors speculate that the steric bulk of the cyclooctyne was preventing the exhaustive modification of the polymer. It

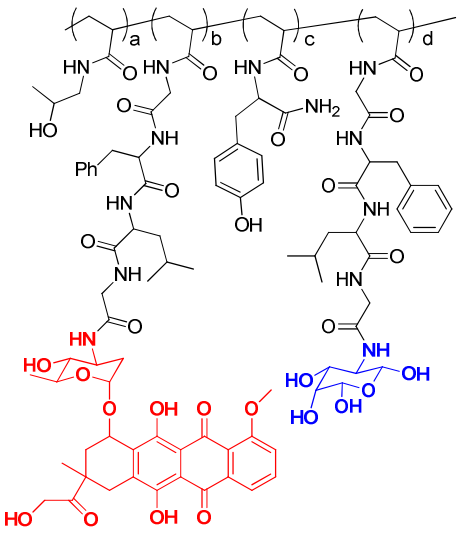
is important to note that ATRP utilizes Cu(I) catalyst for polymerization and the traces of the catalyst may be present in the resulting polymer, making use of copper free conjugation unessential. The SPAAC reaction, although less efficient than CuAAC, is perfectly tailored for post-modification of polymers prepared *via* metal free methods. So far, only few examples of such polymerization-modification sequence were reported. Song *et al.* employed 5,5-bis(azidomethyl)-1,3-dioxan-2-one as the monomer for 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) catalyzed ring opening polymerization to yield and azido-containing polycarbonate. In this report, the functionalization was complete, albeit using three equivalents of the cyclooctyne derivative.[26b] SPAAC was also employed for coupling of cyclooctyne derivative of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) to polymeric nanoparticles prepared by NMP.[26d] In this case, the absence of Cu catalyst was crucial to allow introduction of ^{64}Cu radiolabel for subsequent use in positron emission tomography.

Multi-functional polymers

Attaching several different moieties to a single polymer chain creates a new degree of complexity. Multi-functionalized polymers are promising scaffolds in material and medicinal chemistries.[94] One of many possible applications of multi-functional polymers is drug delivery.[95] Attaching small drug molecules to a polymer scaffold can reduce unwanted cytotoxicity, prolong circulation time, and improve solubility. Polymers also exhibit enhanced permeability and retention (EPR) effect, which helps to target anticancer drugs to a tumor tissue.[96] Multi-functional polymers are especially useful as they can combine sites for drug attachment, targeting, imaging moieties and other functionalities to improve the efficiency of a drug carrier. For example, one of first polymer-based anticancer drugs (PK2) to enter clinical trials had galactosamine residues to provide targeting to the liver and Doxorubicin as a cytotoxic agent

(Scheme 1.11).[97] Comparing to mono-functional polymers discussed in previous section, multi-functional polymers, having two or more orthogonal side chain functionalities, offer a better control of functionalization density.

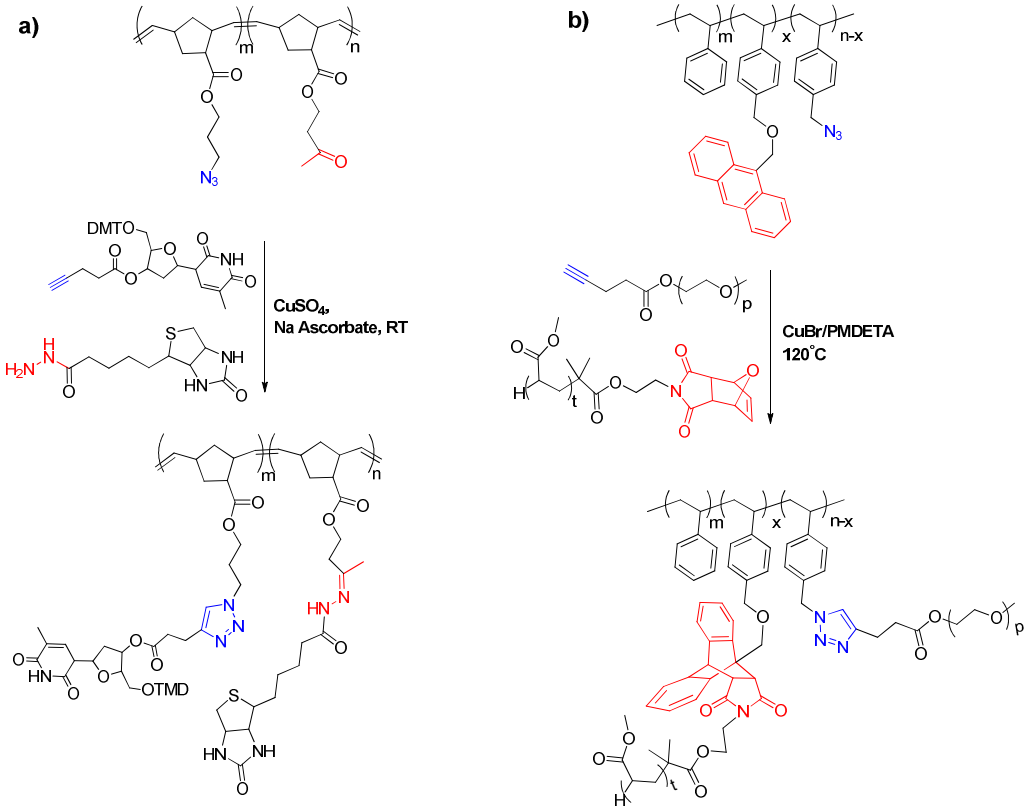
Scheme 1.11 Multi-functional random copolymer polyHPMA-doxorubicin (PK2) used in clinical trials[97]



Recent examples include synthesis of random copolymers containing two orthogonal functional groups for one-pot bi-functionalization. Weck *et al.* reported synthesis of a bi-functional polymeric scaffold using random ruthenium-catalyzed ring-opening copolymerization of *exo*-norbornene esters modified with bromide and ketone moieties (Scheme 1.12a).[98] The azide moiety is known to be incompatible with ROMP conditions due to slow cycloaddition to the double bond of norbornene. The bromide was then displaced with azide to furnish side chain functionality for CuAAC whereas ketone could be independently reacted with various hydrazides. Finally, when alkyne-functionalized nucleoside and biotin hydrazide were mixed with the polymer in presence of the Cu(I) catalyst the one-pot dual functionalization could be

achieved. Tunca *et al.* subsequently reported a preparation of heterograft copolymers using one-pot CuAAC and Diels-Alder coupling (Scheme 1.12b).[99] First, copolymerization of styrene and p-chloromethylstyrene using NMP polymerization afforded a chloride containing copolymer precursor.

Scheme 1.12 Side chain bi-functionalization of random copolymers using orthogonal one-pot reactions. a) SPAAC reaction and hydrazone formation[98] b) SPAAC reaction and Diels-Alder reaction[99]



The chloride group was used to introduce 9-(oxymethyl)antracene ether and azide moieties onto polymeric backbone. This bi-functional polymer was treated with alkyne-terminated PEG and poly(methylmethacrylate) (PMMA) end-functionalized with maleimide at 120°C in presence of CuBr to afford a heterograft terpolymer with PMMA and PEG chains

grafted onto a polystyrene backbone. It was estimated that the grafting efficiency was above 90%, however, the high temperature required for Diels-Alder reaction may impose limitations on types of moieties used for grafting. In material science, the abovementioned polymeric architecture in which various polymer chains are grafted onto core polymer has attracted a significant attention. These polymers are called graft copolymers or molecular brushes.

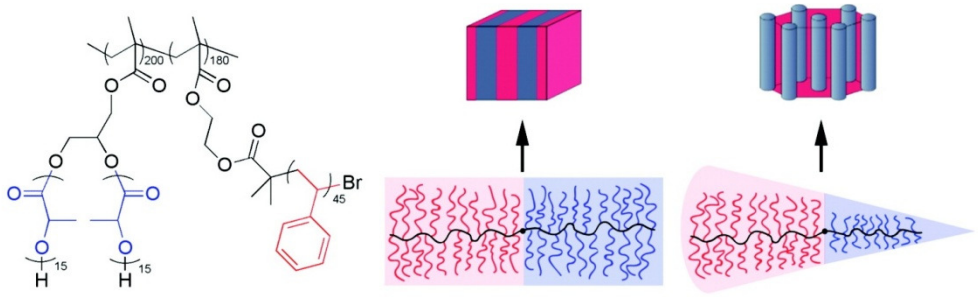


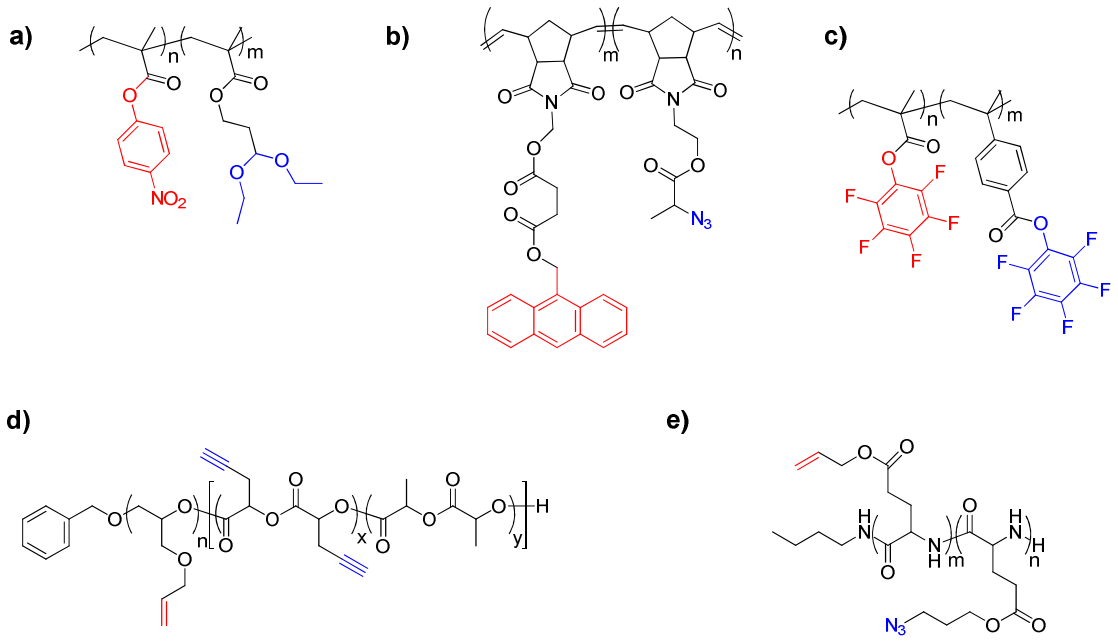
Figure 1.8 Self-assembly of bottlebrush block copolymers with cylindrical morphology. Adapted from reference [100]

Due to steric bulk of polymeric side chains, polymer brushes have extended conformations. This results in a cylindrical shape of a molecule, which leads to fascinating self-assembly properties. Behavior of polymer brushes is largely affected by the nature of side chain polymer grafts. Recently, bi-functional amphiphilic polymer brushes, where the hydrophilic and hydrophobic polymer chains were attached to distinct polymer blocks, were synthesized for the first time.[101] Amphiphilic polymer brushes were shown to self-assemble both in melt and in solution. Interestingly, by varying the ratio of chain length of hydrophilic and hydrophobic blocks, it was possible to change the morphology of self-assembly (Figure 1.8).[100]

Hetero-bi-functional block copolymers with distinct reactive groups in each block can be used to prepare materials self-assembling in solution, bulk or to be used in drug delivery, nanolithography, and preparation of nanomembranes.[102] There are, however, only few reported

methods for the preparation of such polymeric scaffolds since the polymerization has to be living and tolerate the functional groups (Scheme 1.13). Maynard and coworkers employed RAFT polymerization to prepare a bi-functional block copolymer that has activated ester and aldehyde groups amenable for post-polymerization modification (Scheme 1.13a).[103] Successive polymerization of *p*-nitrophenyl methacrylate and diethoxypropyl methacrylate was achieved using dithioester as a chain transfer agent.

Scheme 1.13 Hetero-bi-functional block copolymers[103-104]



The aldehyde moiety was kept protected during the proof of concept functionalization of the activated ester with allylamine, probably to avoid imine formation. The diethyl acetal was then removed to give an aldehyde, which could be functionalized with O-benzylhydroxylamine to give a corresponding oxime. Tunca and coworkers used ROMP to prepare bi-functional block copolymers for sequential CuAAC/Diels-Alder modification (Scheme 1.13b).[104a] This bi-

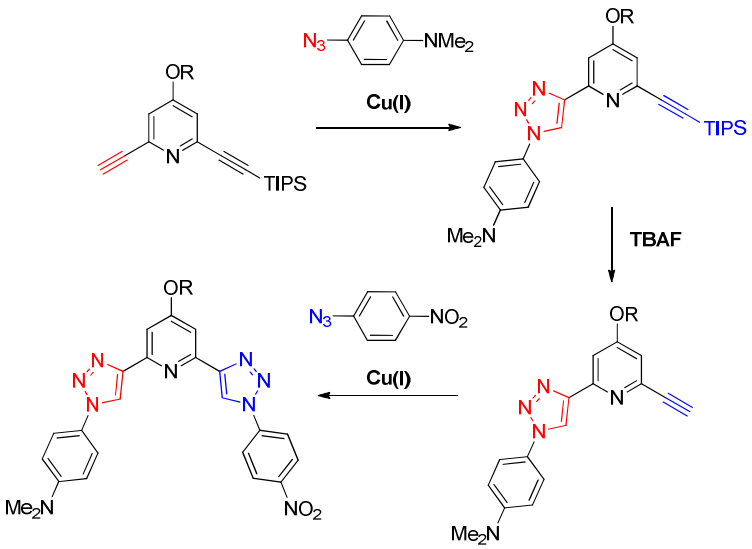
functional block copolymer scaffold to prepare heterograft terpolymers by CuAAC with alkyne-modified poly(ε-caprolactone) and then Diels-Alder reaction with maleimide terminated PMMA. Theato and coworkers used activated esters of different reactivity for sequential modification of polymeric backbone with various amines (Scheme 1.13c).[104b] In particular, the pentafluorophenyl 4-vinylbenzoate reacted with aromatic amines, whether pentafluorophenyl methacrylate was only reactive towards aliphatic amines. Hawker and coworkers designed polylactide-based block copolymer[104c] and Zhang *et al.* reported bi-functional polyglutamic acid copolypeptides[104d] for orthogonal CuAAC and thiol-ene modifications (Scheme 1.13d,e). The main challenges for the construction of such polymers include i) a need for facile introduction of reactive groups onto polymer backbone or tolerance to polymerization conditions; ii) high selectivity and functional group tolerance for post-polymerization modification; iii) limiting the number of reactive species required for post-polymerization modification. These challenges can be addressed using sequential "click" reaction protocols, which will be discussed, in the following section.

Sequential 1,3-dipolar cycloadditions

Once the utility of 1,3-dipolar cycloaddition reactions as conjugation reactions was realized, a search for synthetic methodologies to perform these reactions in successive fashion for multi-functionalization has begun. It is known that silyl-protected alkynes do not undergo CuAAC with azides. TMS group is often used to protect terminal alkynes during polymerizations or other chemical transformations that may affect a triple bond.[105] A repetitive synthetic approach whereby a "click" reaction is followed by deprotection of a silyl-protected alkyne and a second "click" reaction provides an attractive route for the preparation of compounds with complex molecular architectures. Normally, the TMS-deprotection and subsequent CuAAC

reaction are performed in two separate steps. Hence, the "click-click" protocols have been performed in three distinct steps. For example, first, a classical CuAAC is carried out, followed by a silyl-deprotection usually using TBAF or Ag (I) salts and finally another CuAAC is performed with the isolated terminal alkyne. This approach was for example used for construction of multidentate metal ligands "clickates".[106] In a first step, the CuAAC was performed between orthogonally protected 2,6-diethynylpyridines derivative to give a monotriazole (Scheme 1.14).

Scheme 1.14 Preparation of 2,6-bis(1-aryl-1,2,3-tri-azol-4-yl)pyridines (BTPs) using sequential CuAAC reactions[106]

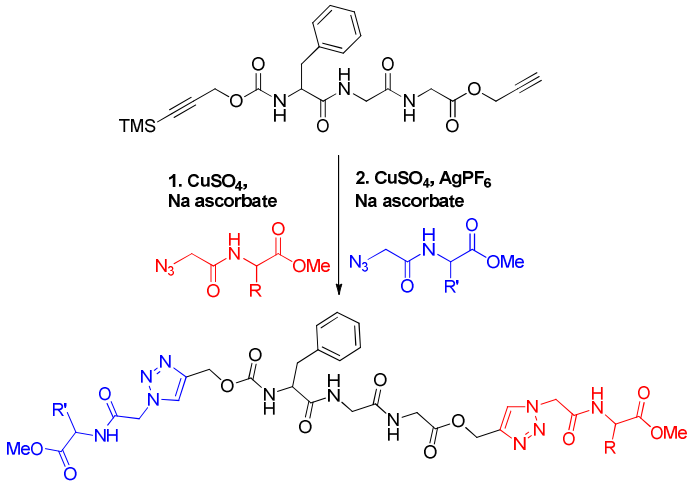


TBAF=tetra(n-butyl)ammonium fluoride).

In a second step the triisopropylsilyl group was removed by treatment with tetra(n-butyl)ammonium fluoride (TBAF). Finally, the second CuAAC step was performed to yield a bis-triazole ligand. It was shown that such "click-click" bi-functionalization could be facilitated by one-pot removal of a silyl protection followed by CuAAC reaction. Aucagne *et al.* reported bi-functionalization of a tripeptide PheGlyGly with two different azido containing amino acids in

only two steps (Scheme 1.15).[107] In this report, a tripeptide esterified with propargyl alcohol at C-terminus and trimethylsilyl propargyl carbamate at N-terminus was prepared. The terminal alkyne of a bi-functional peptide was then reacted with azido-modified amino acid leaving a TMS-protected N-terminal alkyne intact. Next, treatment with silver hexafluorophosphate removed the TMS-group and the second azido-modified amino acid was added to the mixture with additional Cu(I) catalyst.

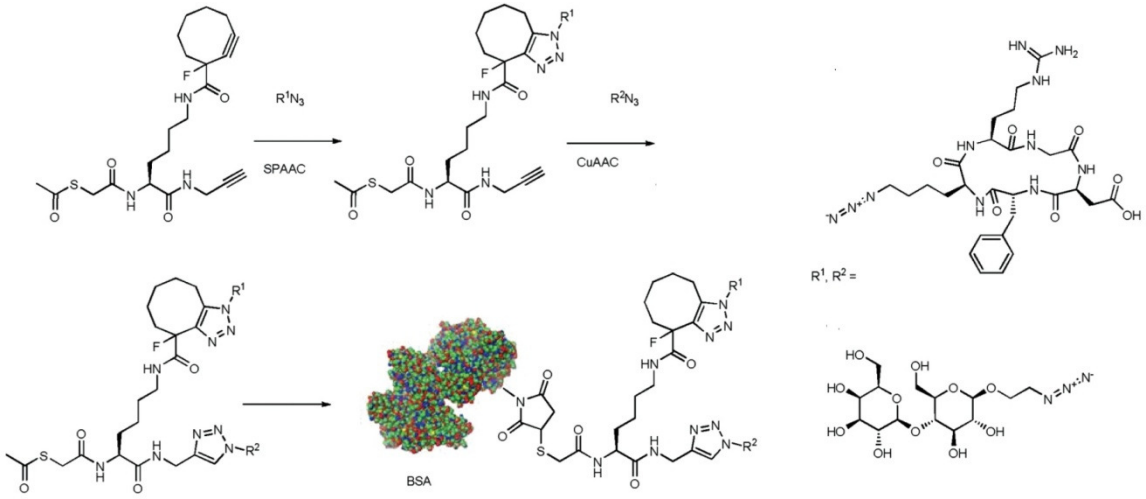
Scheme 1.15 Peptide ligation using sequential CuAAC reactions[107]



This reaction sequence afforded a pseudononapeptide in only two steps. Similar sequential protocols also proved to be useful for oligomerization of amino acids[108], multi-functionalization of fullerenes[109] and construction of rotaxanes.[110] Using the notion that bulky silyl groups such as triisopropylsilyl group (TIPS) are less labile than TMS, triple "click" strategies involving successive CuAAC on terminal, TMS and TIPS protected alkynes were developed for tri-functionalization of small molecules[111] and biomolecules, for example DNA.[112] Strained cyclooctynes react with azides under ambient conditions without need for catalysis.

SPAAC can be performed in presence of terminal alkynes and TMS-protected alkynes. Using this reaction sequence a sequential bi-functionalization of proteins and peptides tagged with cyclooctynes and terminal alkynes was achieved with two different azido-fluorophores to probe FRET effect.[113] Similarly, a hetero-tri-functional template amenable to both copper-free and copper-mediated azide-alkyne cycloadditions and thiol–maleimide coupling to effect sequential biomolecule conjugations in a one-pot process was developed (Scheme 1.16).[114]

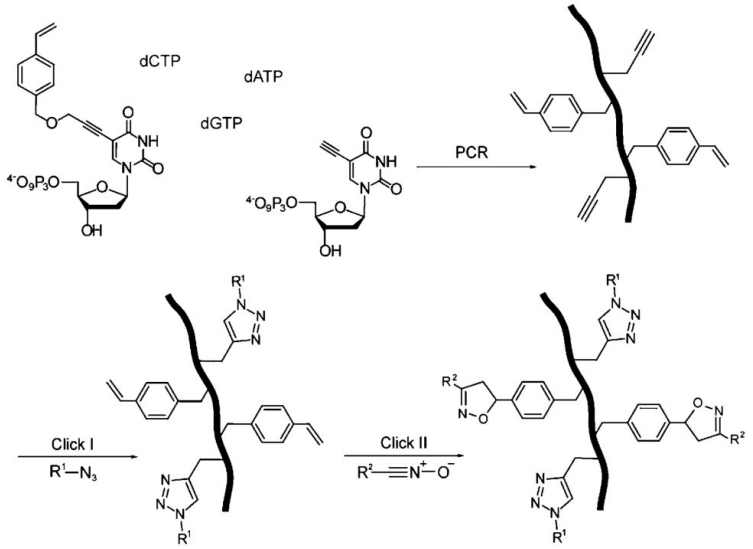
Scheme 1.16 Multi-functionalization using sequential CuAAC and SPAAC reactions. Adapted from reference [114]



This approach allowed for rapid synthesis of an array of bi-functional carbohydrate and peptide conjugates for subsequent protein modification. No protecting groups were required for sugars or peptides when performing sequential cycloadditions, highlighting the utility of this stepwise approach for construction of complex bioconjugates. The above examples took advantage of orthogonal reactivity of terminal, protected, and strained alkynes. Different 1,3-dipoles also exhibit different selectivity towards dipolarophiles.

Nitrile oxides are considerably more reactive in cycloadditions than the azides. They react with number of unactivated alkenes and alkynes whereas azides are not.[115] This allowed sequential bi-functionalization of styrene- and alkyne-modified nucleotides using first CuAAC and then 1,3-dipolar cycloaddition of nitrile oxides to styrene moieties (Scheme 1.17).[116] Both reactions were highly efficient and enable high-density DNA functionalization with oxime-bearing fluorescent tags and azido-sugars.

Scheme 1.17 Multi-functionalization using sequential CuAAC and alkene-nitrile oxide cycloaddition reactions. Adapted from reference [116]



These examples demonstrate that by judicious choice of 1,3-dipoles and dipolarophiles it is possible not only to modify molecular scaffolds with precise control and selectivity, but also to perform multi-functionalization, thereby creating new materials with unique properties. We envisage that sequential 1,3-dipolar cycloadditions could also be used for multi-functionalization of dendrimers and polymers.

Research objective

The common goal of projects described in this dissertation is to expand the synthetic toolbox available to material science for preparation of multi-functional dendrimers and polymers. It is expected that sequential 1,3-dipolar cycloadditions developed for modification of a broad range of molecular structures would also be applicable for functionalization of macromolecular scaffolds. In particular, we anticipate that consecutive CuAAC reactions, utilizing terminal and silyl-protected alkynes, could be used to bi-functionalize the periphery of dendrimers with biologically relevant moieties such as carbohydrates and fluorescent tags. To achieve this goal a novel one-pot procedure for TMS-deprotection/CuAAC reaction, using CuF_2 as a sole reagent, recently developed in our laboratory, will be employed. Since the convergent approach to dendrimer synthesis can yield asymmetric Janus-type dendrimers, we envisage that incorporation of chemoselective SPAAC for linking dendrons with various functionalities could facilitate the synthesis of multi-functional dendrimers. This work is a subject of chapters 2 and 3.

1,3-Dipoles of different reactivity could be used for multi-functionalization of macromolecules. Recently our laboratory has discovered that nitrile oxides can react fast with cyclooctynes. The rate of [3+2] cycloaddition of nitrile oxides was found to be sixty times higher than for analogous reaction with azides. Furthermore, nitrile oxides can be prepared *in situ* from oximes, which are inert during SPAAC reaction. We contemplated that these characteristics of two different 1,3-dipoles could be used for bi-functionalization of various scaffolds containing oxime and azide moieties. The experiments confirmed that SPAAC/SPANOC cycloadditions are a viable synthetic tool for construction of complex glycoconjugates. In order to make this methodology applicable to macromolecular scaffolds, we set off to design a synthetic route to bi-functional block copolymers with pendant azide and oxime groups. The sequential modification

of these polymers with hydrophilic and hydrophobic moieties would yield the amphiphilic polymers with unique self-assembly properties. This work is a subject of chapter 4. The overarching goal of this dissertation is therefore to demonstrate that the use of multiple types of coupling reactions, requiring a diverse set of reagents and conditions is not a necessary requirement for multi-functionalization of macromolecules. The multifaceted nature of 1,3-dipolar cycloadditions allows rapid assembly of diverse macromolecular conjugates from a small set of building blocks bearing alkynes and 1,3-dipoles.

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CHAPTER 2

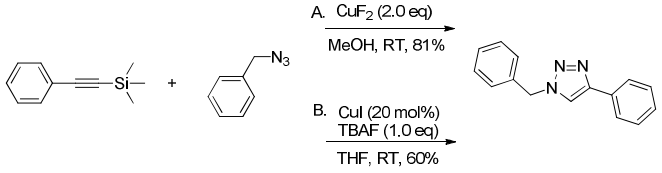
BI-FUNCTIONALIZATION OF DENDRIMERS *VIA* SEQUENTIAL CUAAC REACTIONS

Introduction

Multi-functional macromolecules with two or more types of reactive groups for modification represent an attractive scaffold for creation of new materials. In particular, multi-functionalization allows for precise tuning of molecular properties tailored to a particular function. Dendritic or polymeric macromolecular scaffolds for attachment of various biologically active moieties such as carbohydrates, peptides, nucleotides and drugs have an immense potential in biomedical applications.[1] Unfortunately, traditional coupling reactions require these biomolecules to be protected during attachment to the macromolecular scaffold. Consequently, deprotection steps are usually required after modification. This, however, may affect the integrity of biocompatible macromolecular backbone. Recently, highly chemoselective reactions have been developed which allow for coupling of biomolecules with rich functionality without a need for protecting groups. One of these reactions, Cu(I) catalyzed Azide-Alkyne Cycloaddition (CuAAC), is used extensively for bioconjugation and creation of new materials.[2] As was mentioned earlier, CuAAC has been successfully employed for the synthesis and surface modification of dendrimers.[3] Furthermore, bi-functional dendritic molecules, which have two distinct clusters of functional groups placed at chain ends, are of considerable importance due to their potential ability to combine therapeutic targeting with monitoring. Recently, dendrimers decorated with alkyne functionalities and masked hydroxyl groups have been prepared in an attempt to control further functionalization using sequential CuAAC reactions with azides.[4] Bi-

functionalization, however, requires a set of two orthogonal reactions. It is known that trimethylsilyl (TMS)-protected alkynes do not undergo SPAAC with azides.[5] Consequently, the bi-functional molecule containing two types of alkynes could be functionalized in sequential manner using two different azides. A repetitive synthetic approach where a "click" reaction is followed by deprotection of a silyl-protected alkyne and a second "click" reaction provides an attractive synthetic route for the preparation of compounds with complex molecular architectures[6] and chemoselective modification of biomolecules.[7] Normally, the TMS-deprotection and subsequent CuAAC reaction are performed in two separate steps. Hence, the "click-click" protocols have been performed in three distinct steps. For example, first classical CuAAC is carried out, followed by a silyl-deprotection using usually TBAF or K_2CO_3 and finally another CuAAC is performed with the isolated terminal alkyne.[8] Friscourt *et al.* reported that CuF_2 could be employed in a one-pot two-step procedure, in which the reagent removes a trimethylsilyl protecting group of an alkyne and catalyzed a cycloaddition with an azide.[9] The CuF_2 could deprotect a silyl-alkyne and subsequently catalyze a cycloaddition of the resulting alkyne with an azide to provide the corresponding triazole (Scheme 2.1, condition A.). Although the mechanistic aspects of this transformation are unclear, this approach was found superior to use of a separate desilylating agent (TBAF) and CuI as a catalyst for CuAAC (Scheme 2.1, condition B.).

Scheme 2.1 Synthesis of 1,4-disubstituted-1,2,3-triazoles using a 1-(trimethylsilyl)-2-phenylacetylene and benzyl azide in one-pot conditions

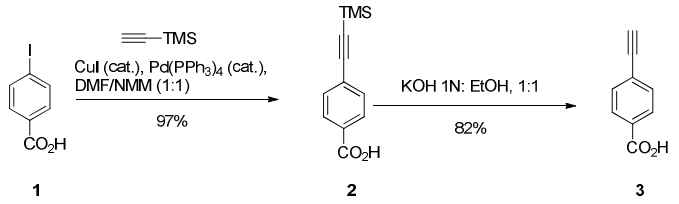


In general, high yields of triazole were obtained when the reaction was performed in polar solvents, and the best results were achieved when THF, DMF or methanol were employed. As expected, at least a stoichiometric quantity of CuF_2 was required to drive the reaction to completion. Furthermore, the CuF_2-mediated process gave high yields of triazole for a variety of different aromatic and aliphatic substituted trimethylsilyl alkynes. Importantly, the CuF_2-mediated one-pot two-step reaction was also compatible with a variety of different azides and for example, the use of substituted phenyl- and benzyl azides, sugar azide and an oligoethylene glycol-modified azide gave the expected products in excellent yields. We envisaged that a one-pot reaction that turns a TMS-protected alkyne into stable triazole would be a perfect fit for sequential bi-functionalization of dendritic scaffolds bearing multiple terminal and TMS-alkynes by tandem CuAAC reactions. The absence of intermediate deprotection steps would facilitate the synthesis of complex glycoconjugates.

Results and Discussion

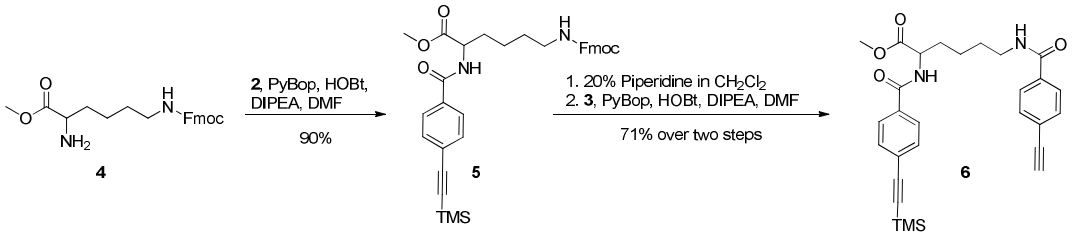
In order to explore the combined use of classical Cu(I)-catalyzed "click" reactions and one-pot two-step reactions mediated by CuF_2 for the preparation of bi-functional compounds a versatile scaffold amenable for introduction of terminal and TMS-protected alkynes was required. We chose a commercially available *N*-ε-Fmoc-L-lysine methyl ester bearing unprotected and Fmoc-protected amines for introduction of two orthogonal functionalities through amide formation. 4-Trimethylsilylethynylbenzoic acid (**2**) was prepared from 4-iodobenzoic acid (**1**) by Sonogashira coupling with trimethylsilylacetylene (Scheme 2.2). Next, 4-ethynylbenzoic acid (**3**) was obtained from **2** by treatment with potassium hydroxide in 82% yield.

Scheme 2.2 Synthesis of 4-trimethylsilylethynylbenzoic acid (**2**) and 4-ethynylbenzoic acid (**3**)



4-Trimethylsilylethynylbenzoic acid (**2**) was then coupled to *N*-ε-Fmoc-L-lysine methyl ester (**4**) using PyBop as a coupling agent to give (**5**) (Scheme 2.3). The Fmoc group was removed by treatment with piperidine in DCM furnishing a free amine (**5a**). (See Experimental Section) Finally, 4-ethynylbenzoic acid (**3**) was coupled with lysine derivative (**5a**) to give a bi-functional scaffold (**6**) for modification *via* sequential CuAAC reactions.

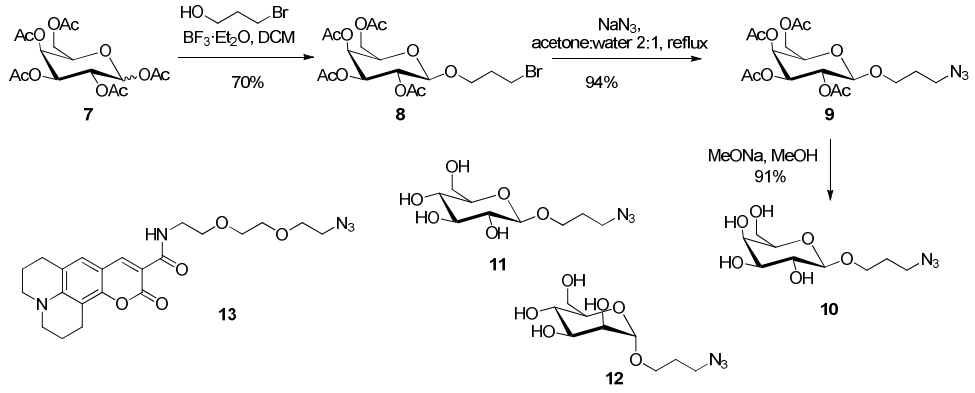
Scheme 2.3 Synthesis of bi-functional scaffold **6**



In order to demonstrate a possibility of sequential CuAAC reactions with various azides on a bi-functional scaffold **6**, unprotected monosaccharide, and fluorescent Coumarin 343 azido-derivatives were prepared. The synthesis of sugar azides was performed in a straightforward fashion (Scheme 2.4). β-D-Galactopyranose pentaacetate (**7**) was glycosylated with 3-bromopropanol in presence of boron trifluoride diethyl etherate to give galactoside **8**. Displacement of bromide with azide was achieved by treatment with sodium azide under reflux conditions. Finally, the 3-azidopropyl β-D-galactopyranoside tetraacetate (**9**) was deacetylated using sodium methoxide to give an unprotected galactoside **10**. 3-Azidopropyl β-D-

glucopyranoside (**11**) and 3-azidopropyl α-D-mannopyranoside (**12**) were synthesized in a similar fashion. (See Experimental Section) Fluorophores represent an important class of moieties in bioconjugation as they allow for quantification and tracking of multi-functional biomedical devices based on polymers and dendrimers. We, therefore, prepared a Coumarin 343 azide (**13**) by coupling of Coumarin 343 carboxylic acid with 2-[2-(2-azidoethoxy)-ethoxy]-ethylamine in presence of PyBop and DIPEA (Scheme 2.4). Having prepared a library of azides of biological significance, we investigated sequential modification of a bi-functional scaffold **6** with unprotected carbohydrates.

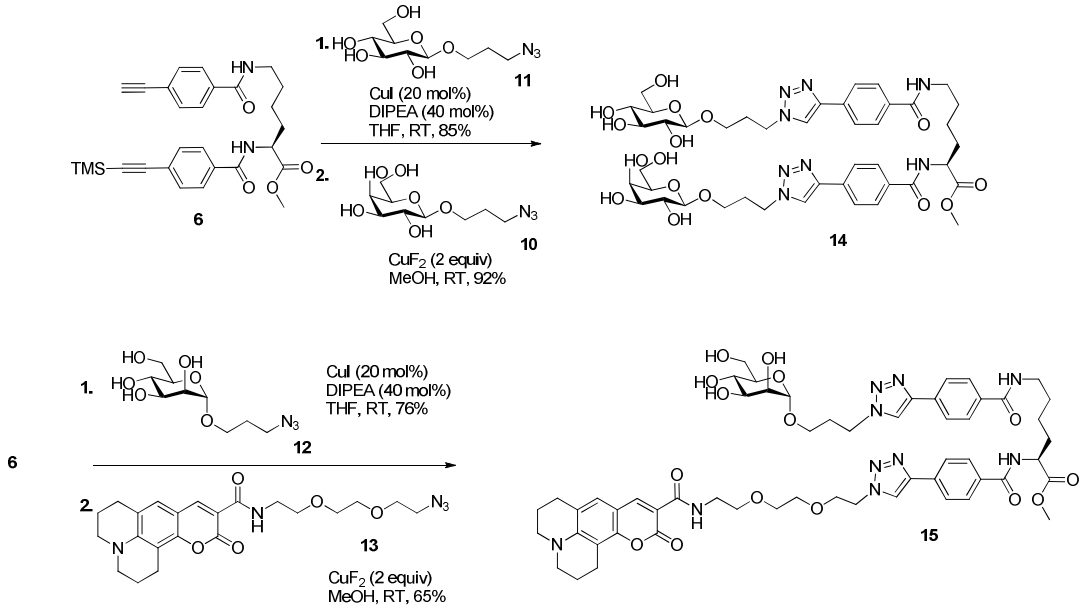
Scheme 2.4 Synthesis of azides **10-13**



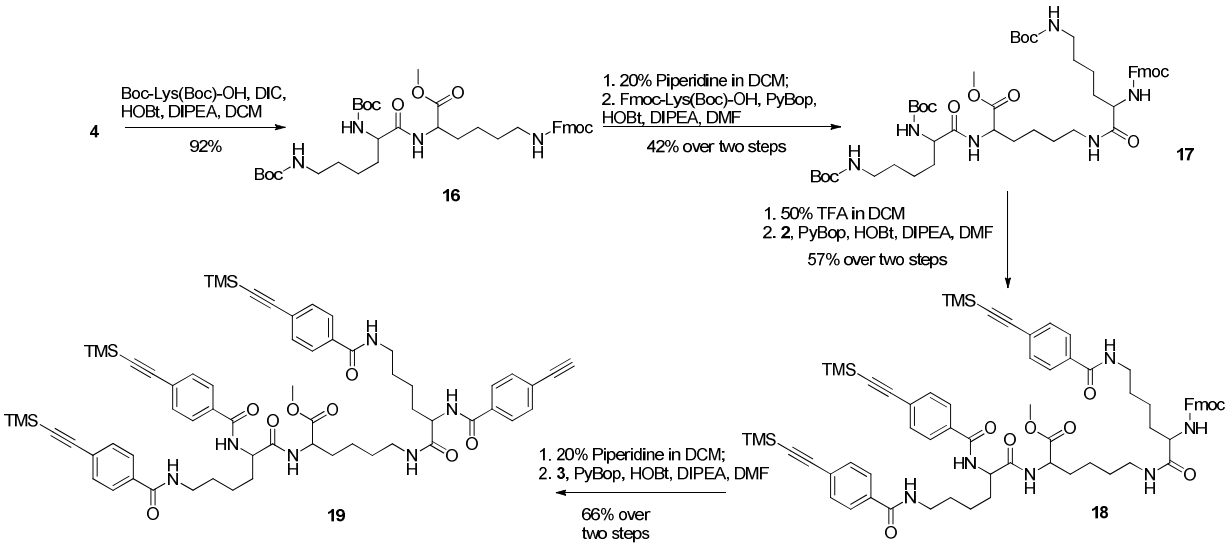
The bi-functional compound **6**, which contains terminal and TMS-protected alkyne moieties, underwent a CuI/DIPEA catalyzed reaction of the terminal alkyne with the 3-azidopropyl β-D-glucopyranoside (**11**) to give the expected triazole **6a** (See Experimental Section) in a yield of 85%. Interestingly, no product was isolated when $CuSO_4$ was employed in the presence of sodium ascorbate in a mixture of water and t-BuOH (1/1, v/v). Next, CuF_2 was employed for the deprotection of the TMS-alkyne moiety and to catalyze the subsequent cycloaddition with 3-azidopropyl β-D-galactopyranoside **10** to give the desired compound **14** in an excellent yield of 92% carrying two different sugars. The use of CuI/TBAF to mediate the

latter reaction led to the isolation of **15** in a lower yield of 67%. A similar strategy was employed for the preparation of bi-functional compound **15** having a mannosyl moiety and a fluorescent label.

Scheme 2.5 Formation of glucosyl-galactosyl derivative **14** and mannosyl-coumarin compound **15** *via* a chemoselective CuAAC/CuAAC sequence

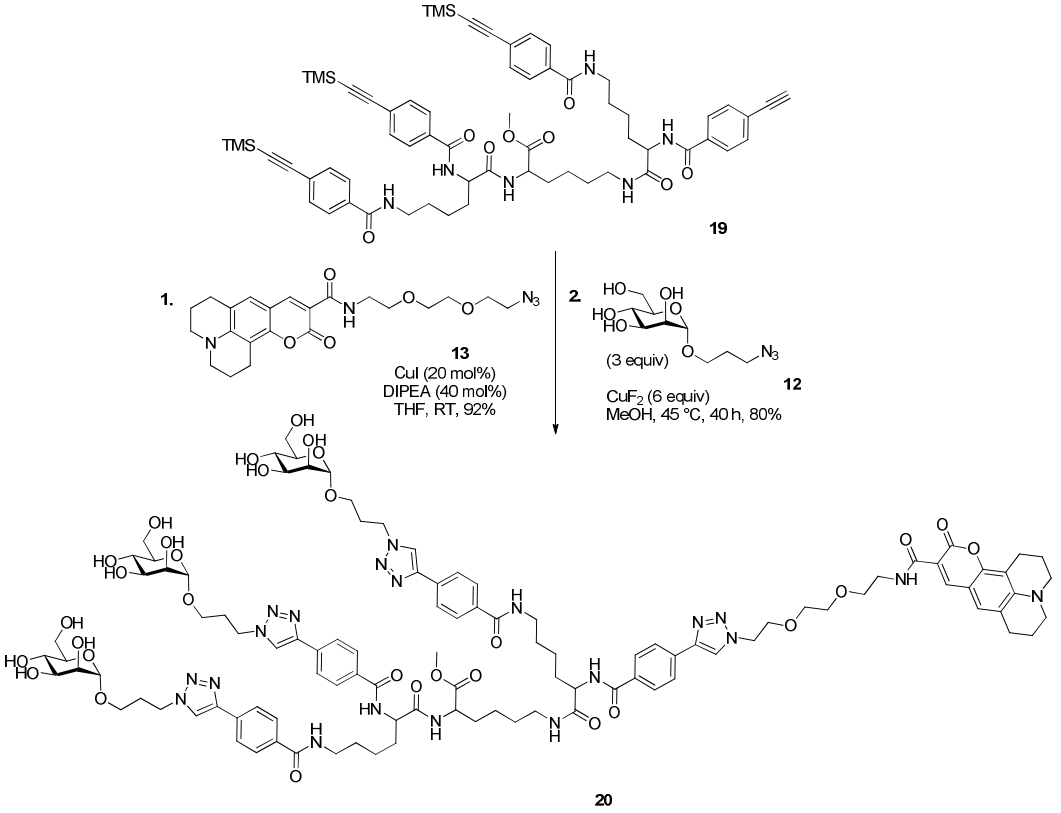


Scheme 2.6 Synthesis of bi-functional dendrimer **19**



Thus, the reaction of scaffold **6** with 3-azidopropyl α-D-mannopyranoside **12** in the presence of CuI/DIPEA in THF gave a mannosylated derivative **6b** (See Experimental Section), which was further modified by a CuF_2-mediated deprotection and "click" reaction with an azido triethylene glycol linked coumarin 343 (**13**) to give compound **15**. We envisaged that a scaffold modified with several terminal- and TMS-protected alkynes would provide an attractive starting material for the facile assembly of multivalent bi-functional compounds. Thus, partially protected *N*-ε-Fmoc-L-lysine methyl ester (**4**) was acylated with N-α,ε-di-t.-Boc-L-lysine in presence of DIC and HOBt to give dipeptide **16**. After removal of the Fmoc group with piperidine the resulting free amine (**16a**) (See Experimental Section) was acylated with *N*-α(Fmoc)-N- ε-t.-Boc-L-lysine using PyBop as coupling agent to give **17**.

Scheme 2.7 Multivalent bi-functionalized dendrimer **20** prepared by chemoselective CuI/CuF_2 "click" reactions



Removal of three Boc groups with 50% TFA in DCM furnished an intermediate (**17a**) (See Experimental Section) with three primary amines. Acylation of these amines with 4-trimethylsilylethynylbenzoic acid (**2**) gave trilysine dendrimer **18** in 74% yield. Finally, the removal of a Fmoc group and subsequent acylation with 4-ethynylbenzoic acid afforded pure bi-functional lysine based dendrimer **19** with 71% yield. Next, scaffold **19** modified with one terminal- and three TMS-protected alkynes was reacted overnight with azido-coumarin 343 (**15**) in the presence of CuI (20 mol%) and DIPEA (40 mol%) in THF at room temperature to give the desired triazole in an excellent yield of 92% (Scheme 2.7). Trimethylsilyl protected alkynes were simultaneously deprotected and clicked with a 3-azido-aminopropanyl mannoside (**12**) employing CuF_2 in methanol at 45 ^oC to give trivalent mannoside **20** in an isolated yield of 80%. The crude reaction mixture was analyzed by HPLC, which showed only the presence of trisubstituted derivative **20**. Bi-functional multivalent derivatives such as **20** offer attractive probes for the investigation of protein-carbohydrate interactions, and in particular, multivalent mannosylated derivatives can be employed to target and detect dendritic cells expressing surface DC-SIGN. This C-type lectin can bind various microorganisms by recognizing high-mannose-containing glycoproteins on their envelopes and has been shown to function as a receptor for several viruses including HIV and Hepatitis C.

Conclusion

Dendritic scaffolds represent a valuable tool for studying carbohydrate-protein interactions due to the multivalent presentation of carbohydrates to lectins.[10] Glycodendrimers can potentially be used as drug delivery vehicles and therefore multi-functionalization to combine several moieties such as targeting, imaging and drug in one macromolecule are often required. Chemoselective CuAAC reactions are often used to derivatize dendrimers with

carbohydrates, however, sequential modification with CuAAC reactions has not been yet reported. Here we demonstrated that a macromolecules can be bi-functionalized using traditional CuAAC followed by a one-pot two-step procedure, in which the CuF_2 reagent removed a trimethylsilyl protecting group of an alkyne and catalyzed a cycloaddition of the resulting terminal alkyne with an azide. The procedure could be employed efficiently for the preparation of several bi-functional model compounds containing unprotected carbohydrates, which can serve as probes to study lectin binding.

Experimental Section

All reactions were carried out under atmosphere of argon, unless stated otherwise. Room temperature refers to ambient room temperature (20-22 °C). Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed silica gel 60 (F254) plates, visualized using UV 254nm and potassium permanganate and ninhydrin dips as appropriate. Flash chromatography was carried out routinely using silica gel G60 (SiliCycle, 60-200μm 60 Å) as the stationary phase unless otherwise stated. The NMR spectra were recorded on a Varian Mercury (300, 500 and 600 MHz) spectrometer. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Coupling constants (*J*) are measured in Hertz (Hz) and are unadjusted; therefore, due to limits in resolution, in some cases there are small differences (<1 Hz) in the measured *J* value of the same coupling constant determined from different signals. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, ddd – doublet of doublet of doublets, tt – triplet of triplets, sp – septet, m – multiplet, br – broad. Various 2D techniques and DEPT experiments were used to establish the structures and to assign the signals. High-resolution mass spectra were obtained by using MALDI-ToF (Applied Biosystems 4700 Proteomics Analyzer)

with 2,5-dihydroxybenzoic acid or α-cyano-4-hydroxycinnamic acid as an internal standard matrix. Reverse Phase HPLC was performed on an Agilent 1200 series system equipped with a manual injector, UV-detector, fraction-collector and Zorbax Eclipse C18 analytical column (5 μm, 9.4 × 250 mm). All solvents were of reagent grade. All chemicals were purchased from Sigma-Aldrich®. CuF_2 (99.5 % purity) were purchased from Alfa Aesar®. Amino acid derivatives and resins were purchased from NovaBioChem and Biosystems.

4-Trimethylsilylethynylbenzoic acid (2). Trimethylsilylacetylene (3.32 mL, 24.0 mmol, 1.2 equiv) was added under argon to a solution of copper (I) iodide (152 mg, 0.8 mmol, 0.04 equiv), 4-iodobenzoic acid (4.96 g, 20.0 mmol, 1.0 equiv) and tetrakis(triphenylphosphine)palladium(0) (462 mg, 0.4 mmol, 0.02 equiv) in THF:NMM (1:1, 20 mL). The reaction mixture was stirred overnight at room temperature. The solvent was then evaporated and the remaining residue was suspended in a water/CH_2Cl_2 mixture (2:1, 100 mL). The mixture was then acidified to pH 3 by addition of 1M hydrochloric acid. The organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (2×30 mL). The organic layers were then combined, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified using flash column chromatography on silica gel with 3% MeOH in CH_2Cl_2 as eluent, affording pure **2** as a yellowish solid (4.22 g, 97%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.27 (s, 9H, $Si(CH_3)_3$), 7.55 (d, J = 8.6 Hz, 2H, H-3,5), 8.05 (d, J = 8.6 Hz, 2H, H-2,6); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -0.19 (3×CH_3, $Si(CH_3)_3$), 98.31 (C, C≡*C*$Si(CH_3)_3$), 103.94 (C, *C*≡C$Si(CH_3)_3$), 128.74 (C), 128.78 (C), 129.99 (2×CH-2,6), 131.93 (2×CH-3,5), 171.93 (C=O) in agreement with the literature data.[11]

4-Ethynylbenzoic acid (3). An aqueous solution of NaOH (1M, 15 mL) was added to a solution of 4-trimethylsilylethynyl benzoic acid **2** (1.74 g, 8.0 mmol) in ethanol (15 mL). The

reaction mixture was stirred overnight at room temperature. The solvent was then evaporated and the remaining residue was suspended in a water/EtOAc mixture (1:1, 100 mL). The pH of the resulting mixture was adjusted to 3 by slow addition of 1M hydrochloric acid and the organic layer was separated. The aqueous layer was further extracted with ethyl acetate (2×30 mL). The organic layers were then combined, dried over $MgSO_4$ and concentrated under vacuum, giving **3** without further purification as a brown solid (960 mg, 82%): ^{1}H NMR (300 MHz, d_6-DMSO) δ 4.43 (s, 1H, C≡C*H*), 7.59 (d, *J* = 8.4 Hz, 2H, H-3,5), 7.93 (d, *J* = 8.4 Hz, 2H, H-2,6); ^{13}C NMR (75.5 MHz, d_6-DMSO) δ 82.64 (CH, C≡*C*H), 83.49 (C, *C*≡CH), 125.91 (C), 129.37 (2×CH-2,6), 130.76 (C), 131.80 (2×CH-3,5), 166.52 (C=O) in agreement with the literature data.[11]

Lysine derivative (5). A solution of 4-Trimethylsilylethynylbenzoic acid **2** (520 mg, 2.4 mmol, 1.2 equiv), PyBop (1.25 g, 2.4 mmol, 1.2 equiv), HOBt (320 mg, 2.4 mmol, 1.2 equiv), DIPEA (700 µL, 4.0 mmol, 2.0 equiv) in dry DMF (10 mL) was stirred for 30 min at room temperature. A solution of *N*-ε-Fmoc-L-lysine methyl ester hydrochloride (836 mg, 2.0 mmol, 1.0 equiv) and DIPEA (700 µL, 4.0 mmol, 2.0 equiv) in dry DMF (5 mL) was then added to the previous mixture. The reaction mixture was then stirred overnight at room temperature. The solvent was evaporated and the crude material was purified by flash chromatography on silica gel using 30% ethyl acetate in hexane, giving pure **5** as a white solid (1.054g, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.27 (s, 9H, $Si(CH_3)_3$), 1.44-1.95 (m, 6H, α-CH(CH_2)$_3$), 3.17-3.23 (m, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 4.18 (t, *J* = 6.5 Hz, 1H, CH(Fmoc)), 4.36 (t, *J* = 6.3 Hz, 2H, CH_2CH(Fmoc)), 4.78 (dd, *J* = 12.2, 7.4 Hz, 1H, α-CH), 4.90 (t, *J* = 5.0 Hz, 1H, NH-Fmoc), 6.82 (d, *J* = 7.1 Hz, 1H, α-NH), 7.29 (t, *J* = 7.5 Hz, 2H, 2×CH(Fmoc)), 7.39 (t, *J* = 7.4 Hz, 2H, 2×CH(Fmoc)), 7.48 (d, *J* = 8.0 Hz, 2H, H-3,5), 7.56 (d, *J* = 7.3 Hz, 2H, 2×CH(Fmoc)), 7.74-7.77 (m, 4H, 2×CH(Fmoc), H-2,6); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -0.16 (3×CH_3, $Si(CH_3)_3$),

22.31 (CH_2, α-$CHCH_2CH_2$), 29.47 (CH_2, CH_2CH_2NH), 31.94 (CH_2, α-$CHCH_2$), 40.25 (CH_2, CH_2NH), 47.20 (CH, CH_2CH(Fmoc)), 52.51 (OCH_3, α-CH), 66.58 (CH_2, CH_2CH(Fmoc)), 97.12 (C, C≡$CSi(CH_3)_3$), 103.96 (C, C≡$CSi(CH_3)_3$), 119.93 (2×CH(Fmoc)), 124.96 (2×CH(Fmoc)), 126.68 (C), 126.99 (2×CH-2,6), 127.00 (2×CH(Fmoc)), 127.63 (2×CH(Fmoc)), 132.02 (2×CH-3,5), 133.20 (C), 141.25 (2×C(Fmoc)), 143.86 (C(Fmoc)), 143.88 (C(Fmoc)), 156.65 (OC=ONH), 166.45 (C=ONH), 172.95 (C=OOMe); HRMS (MALDI) 605.2022 ($C_{34}H_{38}N_2NaO_5Si$ (MNa^+) requires 605.2448).

Unprotected lysine derivative (5a). Compound **5** (1.0 g, 1.7 mmol) was dissolved in a 20% solution of piperidine in CH_2Cl_2 (10 mL). The reaction mixture was stirred for 1 h at room temperature. The solvent was then evaporated and the remaining piperidine was co-evaporated with toluene (3×10 mL). The residue was then passed through a short silica gel column, using 10% MeOH in CH_2Cl_2 as eluent yielding unprotected lysine derivative **21b** as pale yellow oil (576 mg, 93%) which was used in the next step without any further purification and characterization. HRMS (MALDI) 361.1906 ($C_{19}H_{29}N_2O_3Si$ (MH^+) requires 361.1947).

Bi-functional lysine scaffold (6). DIPEA (840μL, 4.8 mmol, 3.0 equiv) was added to a solution of 4-ethynylbenzoic acid **3** (280 mg, 1.9 mmol, 1.2 equiv), PyBop (1.0 g, 1.9 mmol, 1.2 equiv), HOBt (260 mg, 1.9 mmol, 1.2 equiv) in dry DMF (10 mL). The mixture was stirred for 30 min at room temperature. A solution of unprotected lysine derivative **5a** (576 mg, 1.6 mmol, 1.0 equiv) in dry DMF (5mL) was then added to the previous mixture. After overnight stirring, the solvent was evaporated and the residue was purified by flash chromatography on silica gel using 40% ethyl acetate in hexane, affording pure **6** as a white solid (590 mg, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.27 (s, 9H, $Si(CH_3)_3$), 1.50-1.99 (m, 6H, α-$CH(CH_2)_3$), 3.19 (s, 1H, C≡CH), 3.37-3.55 (m, 2H, CH_2NH), 3.77 (s, 3H, OCH_3), 4.78 (td, J = 8.2, 4.5 Hz, 1H, α-CH),

6.45 (t, *J* = 5.0 Hz, 1H, CH_2NH), 6.91 (d, *J* = 7.5 Hz, 1H, α-NH), 7.4-7.50 (m, 4H, H-3,5), 7.67-7.73 (m, 4H, H-2,6); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -0.16 (3×CH_3, $Si(CH_3)_3$), 22.45 (CH_2, α-$CHCH_2CH_2$), 28.67 (CH_2, CH_2CH_2NH), 32.09 (CH_2, α-$CHCH_2$), 39.29 (CH_2, CH_2NH), 52.25 (α-CH), 52.57 (OCH_3), 79.37 (CH, C≡CH), 82.76 (C, C≡CH), 97.20 (C, C≡$CSi(CH_3)_3$), 103.93 (C, C≡$CSi(CH_3)_3$), 125.19 (C), 126.73 (C), 126.90 (2×CH-2,6), 127.00 (2×CH-2,6), 132.03 (2×CH-3,5), 132.17 (2×CH-3,5), 133.19 (C), 134.47 (C), 166.79 (C=ONH), 167.11 (C=ONH), 172.96 (C=O); HRMS (MALDI) 511.1724 ($C_{28}H_{32}N_2NaO_4Si$ (MNa^+) requires 511.2029).

3-Bromopropyl β-D-galactopyranoside tetraacetate (8). Boron trifluoride diethyl etherate (1.92 mL, 15.4 mmol, 2.0 equiv) was added to a solution of β-D-galactopyranose pentaacetate **7** (3 g, 7.7 mmol, 1.0 equiv) and 3-bromopropanol (0.807 µL, 9.24 mmol, 1.2 equiv) in dry CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then poured in a saturated aqueous solution of $NaHCO_3$ (100 mL), the organic layer was separated and the aqueous layer was further extracted with CH_2Cl_2 (3×15 mL). The organic fractions were then combined, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using 30% ethyl acetate in hexane to yield 3-bromopropyl β-D-galactopyranoside tetraacetate **8** as a colorless oil (2.5 g, 70%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.97-2.13 (m, 14H, 4×CH_3, OCH_2CH_2), 3.46 (t, *J* = 5.6 Hz, 2H, $O(CH_2)_2CH_2$), 3.68 (td, *J* = 9.1, 4.3 Hz, 1H, OCH*H*), 3.90 (t, *J* = 6.7 Hz, 1H, H-5), 3.99 (td, *J* = 10.8, 5.8 Hz, 1H, OC*H*H), 4.08-4.20 (m, 2H, H-6), 4.47 (d, *J* = 7.9 Hz, 1H, H-1), 5.01 (dd, *J* = 10.5, 3.4 Hz, 1H, H-3), 5.18 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2), 5.38 (d, *J* = 3.1 Hz, 1H, H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.54 (CH_3), 20.62 (CH_3), 20.64 (CH_3), 20.77 (CH_3), 30.12 (CH_2, OCH_2CH_2), 32.19 (CH_2, $O(CH_2)_2CH_2$), 61.22 (CH_2-6), 66.96 (CH-4), 67.27 (CH_2, OCH_2), 68.80 (CH-2), 70.63 (CH-3), 70.79 (CH-5), 101.51 (CH-1), 169.49

(C=O), 170.07 (C=O), 170.17 (C=O), 170.33 (C=O); HRMS (MALDI) 491.1698 ($C_{17}H_{25}BrNaO_{10}$ (MNa^+) requires 491.0529).

3-Azidopropyl β-D-galactopyranoside tetraacetate (9). Sodium azide (1.74 g, 26.8 mmol, 5.0 equiv) was added to a solution of 3-bromopropyl β-D-galactopyranoside tetraacetate **8** (2.5 g, 5.35 mmol, 1.0 equiv) in an acetone:water mixture (2:1, 20 mL). The reaction mixture was refluxed at 70 °C overnight, then acetone was removed under vacuum and the resulting aqueous solution was extracted with CH_2Cl_2 (3×20 mL). The combined organic fractions were dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using 30% ethyl acetate in hexane to afford 3-azidopropyl β-D-galactopyranoside tetraacetate **9** as a colorless oil (2.18 g, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.74-1.89 (m, 2H, OCH_2CH_2), 1.96-2.15 (m, 12H, 4×CH_3), 3.35 (t, *J* = 6.5 Hz, 2H, $O(CH_2)_2CH_2$), 3.55-3.62 (m, 1H, OCH*H*), 3.87-3.98 (m, 2H, H-5, OC*H*H), 4.07-4.19 (m, 2H, H-6), 4.45 (d, *J* = 7.9 Hz, 1H, H-1), 5.00 (dd, *J* = 10.5, 3.3 Hz, 1H, H-3), 5.18 (dd, *J* = 10.4, 8.0 Hz, 1H, H-2), 5.37 (d, *J* = 3.3 Hz, 1H, H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.78 (CH_3), 20.87 (2×CH_3), 20.95 (CH_3), 29.17 (CH_2, OCH_2CH_2), 48.12 (CH_2, $O(CH_2)_2CH_2$), 61.48 (CH_2-6), 66.66 (CH_2, OCH_2), 67.23 (CH-4), 69.03 (CH-2), 70.90 (CH-3), 71.08 (CH-5), 101.53 (CH-1), 169.63 (C=O), 170.33 (C=O), 170.44 (C=O), 170.58 (C=O); HRMS (MALDI) 454.3684 ($C_{17}H_{25}N_3NaO_{10}$ (MNa^+) requires 454.1438).

3-Azidopropyl β-D-galactopyranoside (10). 3-Azidopropyl β-D-galactopyranoside tetraacetate **9** (2.1 g, 4.87 mmol, 1 equiv) was dissolved in methanol (20 mL) and the pH of the reaction mixture was adjusted to 11 by addition of sodium methoxide. The reaction mixture was stirred for 2 h at room temperature and then was neutralized with addition of Dowex 50WX8-200 ion-exchange resin. The resin was filtered off and the solvent of the filtrate was concentrated

under vacuum. The residue was then purified by flash chromatography on silica gel using 10% methanol in CH_2Cl_2 to yield 3-azidopropyl β-D-galactopyranoside **10** as a colorless oil which solidified upon standing (1.17 g, 91%): ^{1}H NMR (300 MHz, D_2O) δ 1.94 (p, *J* = 6.5 Hz, 2H, OCH_2CH_2), 3.47-3.57 (m, 3H, $O(CH_2)_2CH_2$, H-2), 3.65-3.82 (m, 5H, H-3,5,6, OCH*H*), 3.95 (d, *J* = 3.3 Hz, 1H, H-4), 4.03 (dt, *J* = 10.4, 6.2 Hz, 1H, OC*H*H), 4.42 (d, *J* = 7.8 Hz, 1H, H-1); ^{13}C NMR (75.5 MHz, D_2O) δ 28.43 (CH_2, OCH_2CH_2), 48.07 (CH_2, $O(CH_2)_2CH_2$), 61.12 (CH_2-6), 67.41 (CH_2, OCH_2), 68.80 (CH-4), 70.93 (CH-2), 72.93 (CH-3), 75.30 (CH-5), 103.03 (CH-1); HRMS (MALDI) 286.1162 ($C_9H_{17}N_3NaO_6$ (MNa^+) requires 286.1015).

3-azidopropyl β-D-glucopyranoside **11** and 3-azidopropyl α-D-mannopyranoside **12** were synthesized as described above.

3-azidopropyl β-D-glucopyranoside (11). ^{1}H NMR (300 MHz, D_2O) δ 1.94 (p, *J* = 6.5 Hz, 2H, OCH_2CH_2), 3.29 (t, *J* = 8.5 Hz, 1H, H-2), 3.38-3.55 (m, 5H, H-3,4,5, $O(CH_2)_2CH_2$), 3.71-3.82 (m, 2H, CH*H*-6, OCH*H*), 3.95 (dd, *J* = 12.3, 1.9 Hz, 1H, C*H*H-6), 4.03 (dt, *J* = 10.5, 6.2 Hz, 1H, OC*H*H), 4.48 (d, *J* = 7.9 Hz, 1H, H-1); ^{13}C NMR (75.5 MHz, D_2O) δ 28.41 (CH_2, OCH_2CH_2), 48.06 (CH_2, $O(CH_2)_2CH_2$), 60.92 (CH_2-6), 67.49 (CH_2, OCH_2), 69.83 (CH-4), 73.29 (CH-2), 75.91 (CH-5), 76.09 (CH-3), 102.45 (CH-1); HRMS (MALDI) 286.1214 ($C_9H_{17}N_3NaO_6$ (MNa^+) requires 286.1015).

3-azidopropyl α-D-mannopyranoside (12). ^{1}H NMR (300 MHz, D_2O) δ 1.94 (p, *J* = 6.4 Hz, 2H, OCH_2CH_2), 3.48 (t, *J* = 6.6 Hz, 2H, $O(CH_2)_2CH_2$), 3.60-3.72 (m, 3H, H-4,5, OCH*H*), 3.76-3.99 (m, 5H, H-2,3,6, OC*H*H), 4.89 (d, *J* = 1.4 Hz, 1H, H-1); ^{13}C NMR (75.5 MHz, D_2O) δ 28.02 (CH_2, OCH_2CH_2), 48.38 (CH_2, $O(CH_2)_2CH_2$), 61.08 (CH_2-6), 64.95 (CH_2, OCH_2), 66.90 (CH-4), 70.21 (CH-2), 70.75 (CH-3), 72.94 (CH-5), 99.94 (CH-1); HRMS (MALDI) 286.1912 ($C_9H_{17}N_3NaO_6$ (MNa^+) requires 286.1015).

Azido-Coumarin 343 (13). Coumarin 343 (100 mg, 0.35 mmol, 1.0 equiv), PyBop (182 mg, 0.35 mmol, 1.0 equiv), HOBt (47 mg, 0.35 mmol, 1.0 equiv), DIPEA (61 μL, 0.35 mmol, 1.0 equiv) were dissolved in dry CH_2Cl_2 (2 mL). After the reaction mixture was stirred for 30 min at room temperature, a solution of 2-[2-(2-azidoethoxy)-ethoxy]-ethylamine[12] (61 mg, 0.35 mmol, 1.0 equiv) in dry CH_2Cl_2 (1 mL) was added to the mixture. The reaction mixture was then stirred overnight at room temperature. The solvent was then evaporated and the product was purified by flash chromatography on silica gel using 30 % acetone in hexane, giving pure **13** as a yellow solid (129 mg, 84 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95-1.98 (m, 4H, 2×NCH_2CH_2), 2.76 (t, J = 6.2 Hz, 2H, $N(CH_2)_2CH_2$), 2.87 (t, J = 6.3 Hz, 2H, $N(CH_2)_2CH_2$), 3.29-3.34 (m, 4H, 2×$NCH_2(CH_2)_2$), 3.38-3.41 (m, 2H, CH_2N_3), 3.65-3.72 (m, 10H, 4×OCH_2 , $NHCH_2$), 6.99 (s, 1H, H-vinyl), 8.58 (s, 1H, H-aryl), 9.06 (s, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.09 (CH_2, $N(CH_2)_2CH_2$), 20.19 (CH_2, NCH_2CH_2), 21.13 (CH_2, NCH_2CH_2), 27.43 (CH_2, $N(CH_2)_2CH_2$), 39.38 (CH_2, $NHCH_2$), 49.78 (CH_2, NCH_2), 50.19 (CH_2, NCH_2), 50.71 (CH_2, CH_2N_3), 69.97 (CH_2O), 70.08 (CH_2O), 70.64 (2×CH_2O), 105.64 (C), 108.19 (C), 109.07 (C), 119.52 (C), 126.95 (CH-vinyl), 147.98 (CH-aryl), 148.04 (C), 152.64 (C), 162.85 (C=O), 163.62 (C=O); HRMS (MALDI) 442.2879 ($C_{22}H_{28}N_5O_5$ (MH^+) requires 442.2090).

Glucosyl-lysine derivative (6a). DIPEA (14 μL, 0.08 mmol, 0.4 equiv) was added to a solution of compound **6** (97 mg, 0.2 mmol, 1.0 equiv), 3-azidopropyl β-D-glucopyranoside **11** (52 mg, 0.2 mmol, 1.0 equiv) and CuI (8 mg, 0.04 mmol, 0.2 equiv) in dry THF (2 mL). The resulting mixture was stirred overnight at room temperature. The solvent was then evaporated and the residue was passed through a short silica gel column, using 10% MeOH in CH_2Cl_2 as eluent to afford pure glycosyl-lysine derivative **6a** as a glass-like solid (128 mg, 85%): ^{1}H NMR (300 MHz, d_4-MeOD) δ 0.14 (s, 9H, $Si(CH_3)_3$), 1.36-1.67 (m, 4H, α-$CHCH_2(CH_2)_2$), 1.74-1.95

(m, 2H, α-CHCH_2), 2.15 (p, J = 6.4 Hz, 2H, OCH_2CH_2), 3.11-3.34 (m, 6H, H-2,3,4,5, CH_2NH), 3.45-3.64 (m, 5H, OCH_3, CHH-6, OCHH), 3.78 (dd, J = 11.9, 1.6 Hz, 1H, CHH-6), 3.85 (dt, J = 10.8, 5.6 Hz, 1H, OCHH), 4.18 (d, J = 7.7 Hz, 1H, H-1), 4.48-4.56 (m, 3H, α-CH, $O(CH_2)_2CH_2$), 7.39 (d, J = 8.6 Hz, 2H, H-3'',5''), 7.70 (d, J = 8.6 Hz, 2H, H-2'',6''), 7.75-7.83 (m, 4H, H-2',3',5',6'), 8.39 (s, 1H, H(triazole)); ^{13}C NMR (75.5 MHz, d_4-MeOD) δ -0.08 (3×CH_3, $Si(CH_3)_3$), 24.56 (CH_2, α-$CHCH_2CH_2$), 30.05 (CH_2, CH_2CH_2NH), 31.48 (CH_2, OCH_2CH_2), 31.91 (CH_2, α-$CHCH_2$), 40.64 (CH_2, CH_2NH), 47.20 (CH_2, $O(CH_2)_2CH_2$), 52.81 (OCH_3), 54.42 (α-CH), 62.74 (CH_2-6), 66.98 (CH_2, OCH_2), 71.62 (CH-4), 75.15 (CH-2), 78.03 (CH-3), 78.12 (CH-5), 97.37 (C, $CSi(CH_3)_3$), 104.47 (CH-1), 105.26 (C, $C{\equiv}CSi(CH_3)_3$), 123.76 (CH(triazole)), 126.59 (2×CH-2',6'), 127.86 (C), 128.68 (2×CH-2'',6''), 129.01 (2×CH-3',5'), 132.86 (2×CH-3'',5''), 134.90 (C), 134.97(C), 135.25 (C), 147.71 (C(triazole)), 169.65 (C=ONH), 169.72 (C=ONH), 174.27 (C=O); HRMS (MALDI) 774.4767 ($C_{37}H_{49}N_5NaO_{10}Si$ (MNa^+) requires 774.3146).

Di-functionalized lysine (14). A solution of compound **6a** (50 mg, 0.066 mmol, 1.0 equiv), 3-azidopropyl β-D-galactopyranoside (18 mg, 0.066 mmol, 1.0 equiv) and CuF_2 (13.5 mg, 0.13 mmol, 2.0 equiv) in MeOH (2 mL) was stirred overnight at room temperature. The solvent was then evaporated and the residue was passed through a short silica gel column (Iatrobeads 6RS-8060), using 15% water in acetonitrile as eluent to afford pure di-functionalized lysine **14** as a glass-like solid (57 mg, 92 %): ^{1}H NMR (300 MHz, D_2O) δ 1.39-1.63 (m, 4H, α-$CHCH_2(CH_2)_2$), 1.80-1.97 (m, 2H, α-$CHCH_2$), 2.05-2.19 (m, 4H, 2×OCH_2CH_2), 3.27-3.33 (m, 3H, H-2Glc, CH_2NH), 3.39-3.41 (m, 2H, H-4,5Glc), 3.48-3.75 (m, 12H, H-3Glc, CH-6Glc, H-2,3,5,6Gal, 2×OCHH, OCH_3), 3.82-3.88 (m, 3H, 2×OCHH, CHH-6Glc), 3.94 (d, J = 3.1 Hz, 1H, H-4Gal), 4.32-4.41 (m, 6H, H-1Gal, H-1Glc, 2×$O(CH_2)_2CH_2$), 4.57 (dd, J = 10.2, 4.2 Hz, 1H, α-

CH), 7.24-7.36 (m, 6H, 2×H-3',5', H-2',6'), 7.42 (d, *J* = 8.3 Hz, 2H, H-2',6'), 7.93-7.95 (m, 2H, 2×H(triazole)); ^{13}C NMR (75.5 MHz, D_2O) δ 22.17 (CH_2, α-CHCH$_2$*C*H$_2$), 27.45 (CH_2, *C*H$_2$CH$_2$NH), 29.64 (2×CH_2, 2×OCH$_2$*C*H$_2$), 29.76 (CH_2, α-CH*C*H$_2$), 39.18 (CH_2, *C*H$_2$NH), 47.35 (2×CH_2, 2×O(CH$_2$)$_2$*C*H$_2$), 52.38 (α-CH), 53.09 (OCH_3), 60.88 (CH_2-6Glc), 61.09 (CH_2-6Gal), 66.35 (CH_2, O*C*H$_2$), 66.52 (CH_2, O*C*H$_2$), 68.75 (CH-4Gal), 69.79 (CH-4Glc), 70.95 (CH-2Gal), 72.94 (CH-3Gal), 73.31 (CH-2Glc), 75.23 (CH-5Gal), 75.91 (CH-3Glc), 76.07 (CH-5Glc), 102.41 (CH-1Glc), 102.99 (CH-1Gal), 122.58 (CH(triazole)), 122.65 (CH(triazole)), 125.01 (4×CH-3',5'), 127.42 (2×CH-2',6'), 127.76 (2×CH-2',6'), 132.00 (C), 132.29 (C), 132.65 (C), 132.73 (C), 145.86 (2×C(triazole)), 169.22 (C=ONH), 169.57 (C=ONH), 174.60 (C=O); HRMS (MALDI) 965.3159 ($C_{43}H_{58}N_8NaO_{16}$ (MNa^+) requires 965.3868).

Mannosyl-lysine derivative (6b). DIPEA (14 μL, 0.08 mmol, 0.4 equiv) was added to a solution of compound **6** (97 mg, 0.2 mmol, 1.0 equiv), 3-azidopropyl α-D-mannopyranoside (**12**) (52 mg, 0.2 mmol, 1.0 equiv) and CuI (8 mg, 0.04 mmol, 0.2 equiv) in dry THF (2 mL). The resulting mixture was stirred overnight at room temperature. The solvent was then evaporated and the residue was passed through a short silica gel column, using 10% MeOH in CH_2Cl_2 as eluent to afford pure mannosyl-lysine derivative **6b** as a glass-like solid (114 mg, 76%): ^{1}H NMR (300 MHz, *d*$_4$-MeOD) δ 0.19 (s, 9H, Si(CH$_3$)$_3$), 1.43-1.53 (m, 2H, α-CHCH$_2$C*H*$_2$), 1.61-1.69 (m, 2H, C*H*$_2$CH$_2$NH), 1.79-2.02 (m, 2H, α-CHC*H*$_2$), 2.21 (p, *J* = 6.5 Hz, 2H, OCH$_2$C*H*$_2$), 3.34-3.68 (m, 10H, C*H*$_2$NH, OCH*H*, H-3,4,5, OCH_3, CH*H*-6), 3.73-3.80 (m, 3H, OC*H*H, H-2, C*H*H-6), 4.52-4.58 (m, 3H, α-CH, O(CH$_2$)$_2$C*H*$_2$), 4.70 (d, *J* = 1.4 Hz, 1H, H-1), 7.43 (d, *J* = 8.4 Hz, 2H, H-3'',5''), 7.75 (d, *J* = 8.4 Hz, 2H, H-2'',6''), 7.80-7.88 (m, 4H, H-2',3',5',6'), 8.39 (s, 1H, H(triazole)); ^{13}C NMR (75.5 MHz, *d*$_4$-MeOD) δ -0.09 (3×CH_3, Si(CH$_3$)$_3$), 24.57 (CH_2, α-CHCH$_2$*C*H$_2$), 30.06 (CH_2, *C*H$_2$CH$_2$NH), 31.31 (CH_2, OCH$_2$*C*H$_2$), 31.91 (CH_2, α-CH*C*H$_2$), 40.63

(CH_2, *C*H_2NH), 48.88 (CH_2, O(CH_2)$_2$*C*H_2), 52.81 (OCH_3), 54.42 (α-CH), 62.96 (CH_2-6), 65.24 (CH_2, O*C*H_2), 68.65 (CH-4), 72.11 (CH-2), 72.67 (CH-3), 74.89 (CH-5), 97.37 (C, *C*Si(CH_3)$_3$), 101.85 (CH-1), 105.26 (C, *C*≡CSi(CH_3)$_3$), 123.25 (CH(triazole)), 126.63 (2×CH-2',6'), 127.88 (C), 128.69 (2×CH-2'',6''), 129.02 (2×CH-3',5'), 132.86 (2×CH-3'',5''), 134.85 (C), 134.98 (C), 135.29 (C), 147.90 (C(triazole)), 169.67 (C=ONH), 169.73 (C=ONH), 174.29 (C=O); HRMS (MALDI) 774.5560 ($C_{37}H_{49}N_5NaO_{10}Si$ (MNa^+) requires 774.3146).

Monovalent mannose probe (15). A solution of compound **6b** (55 mg, 0.1 mmol, 1 equiv), coumarin-azide **13** (44 mg, 0.1 mmol, 1 equiv) and CuF_2 (20 mg, 0.2 mmol, 2 equiv) in MeOH (2 mL) was stirred overnight at room temperature. The solvent was then evaporated and the residue was passed through a short silica gel column, using 10% MeOH in CH_2Cl_2 as eluent to afford pure monovalent mannose probe **13** as a yellow solid (57 mg, 65 %): ^{1}H NMR (500 MHz, d_6-DMSO+D_2O) δ 1.38-1.49 (m, 2H, α-CHCH$_2$C*H*$_2$), 1.53-1.60 (m, 2H, α-CH(CH$_2$)$_2$C*H*$_2$), 1.84-1.85 (m, 6H, α-CHC*H*$_2$, 2×NCH$_2$C*H*$_2$), 2.14 (p, *J* = 6.4 Hz, 2H, OCH$_2$C*H*$_2$CH$_2$), 2.67 (t, *J* = 5.7 Hz, 4H, 2×N(CH$_2$)$_2$C*H*$_2$), 3.26-3.68 (m, 24H, α-CH(CH$_2$)$_3$C*H*$_2$, 2×NC*H*$_2$, H-2,3,4,5, C*H*H-6, OC*H*$_2$(CH$_2$)$_2$, NHC*H*$_2$CH$_2$O, NHCH$_2$C*H*$_2$O(C*H*$_2$)$_2$O, OCH_3), 3.89 (t, *J* = 5.1 Hz, 2H, triazole-CH$_2$C*H*$_2$O), 4.40-4.52 (m, 3H, α-CH, O(CH$_2$)$_2$C*H*$_2$-triazole), 4.57-4.61 (m, 3H, OCH$_2$C*H*$_2$-triazole, H-1), 7.19 (s, 1H, H-vinyl), 7.87-7.95 (m, 8H, H-2',3',5',6'), 8.47 (s, 1H, H-aryl), 8.51 (t, *J* = 5.3 Hz, 1H, CH$_2$N*H*), 8.58 (s, 1H, H(triazole)), 8.65 (s, 1H, H(triazole)), 8.73 (d, *J* = 7.2 Hz, 1H, α-CHN*H*), 8.80 (t, *J* = 5.5Hz, N*H*(CH$_2$)$_2$O); ^{13}C NMR (75.5 MHz, d_6-DMSO+D_2O) δ 19.46 (2×CH_2, NCH$_2$*C*H$_2$, N(CH$_2$)$_2$*C*H$_2$), 20.42 (CH_2, NCH$_2$*C*H$_2$), 23.16 (CH_2, α-CHCH$_2$*C*H$_2$), 26.69 (CH_2, N(CH$_2$)$_2$*C*H$_2$), 28.62 (CH_2, α-CH(CH$_2$)$_2$*C*H$_2$), 29.71 (CH_2, OCH$_2$*C*H$_2$), 30.08 (CH_2, α-CH*C*H$_2$), 38.69 (NH*C*H$_2$CH$_2$O), 38.80 (CH_2, α-CH(CH$_2$)$_3$*C*H$_2$), 46.96 (CH_2, O(CH$_2$)$_2$*C*H$_2$), 48.89 (NCH_2), 49.43 (CH_2, triazole-CH_2), 49.62 (CH_2, NCH_2), 51.76 (OCH_3), 52.69 (α-CH),

61.14 (CH_2-6), 63.18 (CH_2, O*C*$H_2(CH_2)_2$), 66.91 (CH-4), 68.59 (triazole-CH_2*C*H_2O), 68.99 (CH_2O), 69.47 (CH_2O), 69.57 (CH_2O), 70.18 (CH-2), 70.95 (CH-3), 74.05 (CH-5), 99.90 (C), 104.49 (CH-1), 107.27 (C), 107.60 (C), 119.31 (C), 122.09 (CH(triazole)), 122.47 (CH(triazole)), 124.63 (2×CH-3',5'), 124.67 (2×CH-3',5'), 127.02 (CH-vinyl), 127.75 (2×CH-2',6'), 128.10 (2×CH-2',6'), 132.59 (C), 133.16 (C), 133.55 (C), 133.62 (C), 145.32 (C), 145.52 (C), 147.42 (C), 147.88 (CH-aryl), 151.96 (C), 161.81 (C=O), 162.39 (C=O), 165.63 (C=ONH), 166.14 (C=ONH), 172.75 (C=OOCH_3); HRMS (MALDI) 1143.7560 ($C_{56}H_{68}N_{10}NaO_{15}$ (MNa^+) requires 1143.4758).

Dilysine derivative (16). A solution of N-α,ε-di-t.-Boc-L-lysine (1.35 g, 6.8 mmol, 1.2 equiv), *N,N′*-diisopropylcarbodiimide (1.05 mL, 6.8 mmol, 1.2 equiv), HOBt (918 mg, 6.8 mmol, 1.2 equiv) and DIPEA (1.95 mL, 11.2 mmol, 2.0 equiv) in dry DMF (20 mL) was stirred for 20 min at room temperature. When the urea precipitate had formed, a solution of N- ε(Fmoc)-L-lysine methyl ester hydrochloride (2.34 g, 5.6 mmol, 1.0 equiv) and DIPEA (1.95 mL, 11.2 mmol, 2.0 equiv) in dry DMF (10 mL) was added. The reaction mixture was then stirred overnight at room temperature. The mixture was then filtered and the resulting filtrate was concentrated under vacuum. The residue was then dissolved in ethyl acetate (50 mL), washed with aq. $NaHCO_3$ (2×100 mL) and water (100 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude was then purified by flash chromatography on silica gel using 30% acetone in hexane to give pure **16** as a white solid (3.61 g, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35-1.86 (m, 30H, 2×α-CH(C*H*$_2$)$_3$, 2×$(CH_3)_3$C), 3.03-3.21 (m, 4H, 2×C*H*$_2$-NH), 3.73 (s, 3H, OCH_3), 4.09-4.11 (m, 1H, α-CH), 4.21 (t, *J* = 7.0 Hz, 1H, CH(Fmoc)), 4.41 (d, *J* = 7.4 Hz, 2H, C*H*$_2$CH(Fmoc)), 4.53-4.66 (m, 2H, α-CH, NH), 5.09 (s, 1H, NH), 5.21 (s, 1H, NH), 6.73 (d, *J* = 7.1 Hz, 1H, NH), 7.30 (td, *J* = 7.4, 1.0 Hz, 2H, CH(Fmoc)), 7.39 (t, *J* = 7.3 Hz, 2H, CH(Fmoc)),

7.61 (d, *J* = 7.4 Hz, 2H, CH(Fmoc)), 7.76 (d, *J* = 7.4 Hz, 2H, CH(Fmoc)); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.28 (CH_2, α-$CHCH_2\mathit{C}H_2$), 22.41 (CH_2, α-$CHCH_2\mathit{C}H_2$), 28.26 (3×CH_3, $(\mathit{C}H_3)_3C$), 28.37 (3×CH_3, $(\mathit{C}H_3)_3C$), 29.14 (CH_2, $\mathit{C}H_2CH_2NH$), 29.50 (CH_2, $\mathit{C}H_2CH_2NH$), 31.56 (CH_2, α-$CH\mathit{C}H_2$), 31.94 (CH_2, α-$CH\mathit{C}H_2$), 39.81 (CH_2, $\mathit{C}H_2NH$), 40.32 (CH_2, $\mathit{C}H_2NH$), 47.20 (CH, $CH_2\mathit{C}H$(Fmoc)), 51.90 (α-CH), 52.31 (OCH_3), 54.23 (α-CH), 66.48 (CH_2, $\mathit{C}H_2CH$(Fmoc)), 78.96 (C, $(CH_3)_3\mathit{C}$), 79.92 (C, $(CH_3)_3\mathit{C}$), 119.87 (2×CH(Fmoc)), 125.01 (2×CH(Fmoc)), 126.95 (2×CH(Fmoc)), 127.58 (2×CH(Fmoc)), 141.21 (2×C(Fmoc)), 143.89 (C(Fmoc)), 143.94 (C(Fmoc)), 155.74 (C=ONH), 156.08 (C=ONH), 156.57 (C=ONH), 172.27 (C=O), 172.50 (C=O); HRMS (MALDI) 733.3958 ($C_{38}H_{54}N_4NaO_9$ (MNa^+) requires 733.3788).

Unprotected Dilysine (16a). Compound **16** (3.60 g, 5.07 mmol) was dissolved in 20% solution of piperidine in CH_2Cl_2 (20 mL). The mixture was stirred for 1 h at room temperature, the solvent was then evaporated and the remaining piperidine was co-evaporated with toluene (3×20 mL). The residue was passed through a short silica gel column using 10% MeOH in CH_2Cl_2 as eluent to give **16a** as colorless oil which was used in the next step without any further purification and full characterization (1.37g, 56%): HRMS (MALDI) 511.3063 ($C_{23}H_{44}N_4NaO_7$ (MNa^+) requires 511.3108).

Trilysine derivative (17). A solution of N- α(Fmoc)-N- ε-t.-Boc-L-lysine (1.55 g, 3.32 mmol, 1.2 equiv), PyBop (1.73 g, 3.32 mmol, 1.2 equiv), HOBt (450 mg, 3.32 mmol, 1.2 equiv) and DIPEA (1.34 mL, 8.31 mmol, 3.0 equiv) in dry DMF (20 mL) was stirred for 30 min at room temperature. A solution of compound **16b** (1.35 g, 2.77 mmol, 1.0 equiv) in dry DMF (10 mL) was then added to the reaction mixture and the mixture was stirred overnight at room temperature. The solvent was then removed under vacuum and the residue was dissolved in ethyl acetate (50 mL), washed with aq. $NaHCO_3$ (2×100 mL) and water (100 mL), dried over $MgSO_4$

and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel using 40% acetone in hexane to afford pure **17** as a white solid (2.00 g, 77%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24-1.85 (m, 45H, 3×α-CH(C*H*$_2$)$_3$, 3×(CH$_3$)$_3$C), 2.94-3.10 (m, 5H, 2×C*H*$_2$NH, CH*H*NH), 3.42-3.55 (m, 1H, C*H*HNH), 3.66 (s, 3H, OCH$_3$), 4.13 (t, *J* = 7.2 Hz, 1H, CH(Fmoc)), 4.23-4.37 (m, 5H, 3×α-CH, C*H*$_2$CH(Fmoc)), 4.81-4.88 (m, 2H, 2×CH$_2$N*H*), 5.78 (d, *J* = 6.4 Hz, 1H, α-NH), 6.38 (d, *J* = 6.3 Hz, 1H, α-NH), 7.24-7.39 (m, 5H, 4×CH(Fmoc), CH$_2$N*H*), 7.52-7.62 (m, 3H, 2×CH(Fmoc), α-NH), 7.72 (d, *J* = 7.5 Hz, 2H, 2×CH(Fmoc)); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 22.46 (2×CH$_2$, 2×α-CHCH$_2$*C*H$_2$), 22.75 (CH$_2$, α-CHCH$_2$*C*H$_2$), 28.28 (3×CH$_3$, (*C*H$_3$)$_3$C), 28.38 (6×CH$_3$, 2×(*C*H$_3$)$_3$C), 28.59 (CH$_2$, *C*H$_2$CH$_2$NH), 29.28 (CH$_2$, *C*H$_2$CH$_2$NH), 29.48 (CH$_2$, *C*H$_2$CH$_2$NH), 31.06 (CH$_2$, α-CH*C*H$_2$), 32.18 (CH$_2$, α-CH*C*H$_2$), 32.35 (CH$_2$, α-CH*C*H$_2$), 38.16 (CH$_2$, *C*H$_2$NH), 40.00 (CH$_2$, *C*H$_2$NH), 40.17 (CH$_2$, *C*H$_2$NH), 46.90 (CH, CH$_2$*C*H(Fmoc)), 52.17 (OCH$_3$), 52.28 (α-CH), 53.86 (α-CH), 54.46 (α-CH), 67.34 (CH$_2$, *C*H$_2$CH(Fmoc)), 78.75 (C, (CH$_3$)$_3$*C*), 78.89 (C, (CH$_3$)$_3$*C*), 79.88 (C, (CH$_3$)$_3$*C*), 119.86 (2×CH(Fmoc)), 125.11 (2×CH(Fmoc)), 127.07 (2×CH(Fmoc)), 127.67 (2×CH(Fmoc)), 141.12 (2×C(Fmoc)), 143.59 (C(Fmoc)), 143.72 (C(Fmoc)), 155.99 (2×C=ONH), 156.20 (C=ONH), 156.88 (C=ONH), 172.42 (C=O), 172.80 (C=O), 173.41 (C=O); HRMS (MALDI) 961.6054 ($C_{49}H_{74}N_6NaO_{12}$ (MNa^+) requires 961.5262).

Unprotected Trilysine derivative (17b). Compound **17** (2 g, 2.13 mmol) was dissolved in a 50% solution of TFA in CH_2Cl_2 (20 mL). The mixture was stirred for 2 h at room temperature. The solvent was then evaporated and the crude product was precipitated by addition of diethyl ether (70 mL) and collected by filtration as a white solid which was used in the next step without any further purification and full characterization (1.54 g. 78%): HRMS (MALDI) 639.3571 ($C_{34}H_{51}N_6O_6$ (MH^+) requires 639.3870).

TMS-Trilysine derivative (18). A solution of 4-trimethylsilylethynylbenzoic acid **2** (1.43 g, 6.56 mmol, 4 equiv), PyBop (3.42 g, 6.57 mmol, 4 equiv), HOBt (1.10 g, 6.56 mmol, 4 equiv) and DIPEA (1.14 mL, 6.56 mmol, 4 equiv) in dry DMF (15 mL) was stirred for 30 min at room temperature. A solution of TFA salt of deprotected trilysine derivative **17b** (1.54 g, 1.64 mmol, 1 equiv) and DIPEA (1.14 mL, 6.56 mmol, 4 equiv) in dry DMF (10 mL) was then added to the reaction mixture. The latter mixture was stirred for 24 h at room temperature and then concentrated under vacuum. The residue was dissolved in ethyl acetate (60 mL), washed with aq. $NaHCO_3$ (2×150 mL) and water (2×150 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel using 3% MeOH in CH_2Cl_2 to yield pure **18** as a white solid (1.49 g, 74 %): ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.24 (s, 27H, 3×Si(CH_3)$_3$), 1.22-1.80 (m, 18H, 3×α-CH(CH_2)$_3$), 2.97-3.10 (m, 2H, CH_2NH), 3.17-3.29 (m, 4H, 2× CH_2NH), 3.60 (s, 3H, OCH_3), 3.92 (q, J = 8.2 Hz, 1H, α-CH), 4.15-4.27 (m, 4H, C*H*(Fmoc), α-CH, CH_2CH(Fmoc)), 4.47 (q, J = 7.5 Hz, 1H, α-CH), 7.30 (t, J = 7.5 Hz, 2H, 2×CH(Fmoc)), 7.38-7.43 (m, 3H, 2×CH(Fmoc), α-NH), 7.50-7.53 (m, 6H, 3×H-3,5), 7.71 (d, J = 7.3 Hz, 2H, 2×CH(Fmoc)), 7.80-7.89 (m, 8H, 2×CH(Fmoc), 3×H-2,6), 8.32 (d, J = 7.3 Hz, 1H, α-NH), 8.48-8.56 (m, 3H, 2×CH_2N*H*, α-NH); ^{13}C NMR (75.5 MHz, d_6-DMSO) δ -0.28 (9×CH_3, 3×Si(CH_3)$_3$), 22.57 (CH_2, α-CHCH$_2$*C*H$_2$), 22.98 (CH_2, α-CHCH$_2$*C*H$_2$), 23.10 (CH_2, α-CHCH$_2$*C*H$_2$), 28.50 (CH_2, *C*H$_2$CH$_2$NH), 28.66 (CH_2, *C*H$_2$CH$_2$NH), 28.75 (CH_2, *C*H$_2$CH$_2$NH), 30.32 (CH_2, α-CH*C*H$_2$), 31.16 (CH_2, α-CH*C*H$_2$), 31.70 (CH_2, α-CH*C*H$_2$), 38.09 (CH_2, *C*H$_2$NH), 39.02 (2×CH_2, 2×*C*H$_2$NH), 46.57 (CH, CH$_2$*C*H(Fmoc)), 51.62 (OCH_3), 51.85 (α-CH), 53.11 (α-CH), 54.56 (α-CH), 65.47 (CH_2, *C*H$_2$CH(Fmoc)), 96.13 (2×C, 2×*C*Si(CH_3)$_3$), 96.26 (C, *C*Si(CH_3)$_3$), 104.38 (3×C, 3×*C*≡CSi(CH_3)$_3$), 119.96 (2×CH(Fmoc)), 124.50 (C), 124.73 (C), 125.21 (2×CH(Fmoc)), 126.91 (2×CH(Fmoc)), 127.31 (6×CH-2,6), 127.49 (C),

127.70 (2×CH(Fmoc)), 131.28 (2×CH-3,5), 131.33 (4×CH-3,5), 134.02 (C), 134.52 (2×C), 140.59 (2×C(Fmoc)), 143.69 (C(Fmoc)), 143.78 (C(Fmoc)), 155.80 (C=ONH), 165.07 (C=ONH), 165.11 (C=ONH), 165.47 (C=ONH), 171.64 (C=O), 172.07 (C=O), 172.41 (C=O); HRMS (MALDI) 1261.4893 ($C_{70}H_{86}N_6NaO_9Si_3$ (MNa^+) requires 1261.5662).

Unprotected trilysine-TMS-Phenyl acetylene derivative (18a). Compound **18** (1.49 g, 1.2 mmol) was dissolved in a 20% solution of piperidine in CH_2Cl_2 (15 mL). The mixture was stirred for 2 h at room temperature. The solvent was then evaporated and the remaining piperidine was co-evaporated with toluene (3×20 mL). The residue was then passed through a short silica gel column, using 10% MeOH in CH_2Cl_2 as eluent to yield pale yellow oil, which was used in the next step without any further purification and full characterization (1.12 g, 92 %). HRMS (MALDI) 1039.4895 ($C_{55}H_{76}N_6NaO_7Si_3$ (MNa^+) requires 1039.4981).

Trilysine scaffold (19). A solution of 4-ethynylbenzoic acid (**2**) (241 mg, 1.65 mmol, 1.5 equiv), PyBop (858 mg, 1.65 mmol, 1.5 equiv), HOBt (223 mg, 1.65 mmol, 1.5 equiv) and DIPEA (380 μL, 2.20 mmol, 2.0 equiv) in dry DMF (15 mL) was stirred for 30 min at room temperature. A solution of unprotected compound **18a** (1.12 g, 1.10 mmol, 1.0 equiv) in dry DMF (10 mL) was then added to the latter reaction mixture. The mixture was then stirred overnight at room temperature and concentrated under vacuum. The residue was then dissolved in ethyl acetate (70 mL), washed with aq. $NaHCO_3$ (2×100 mL) and water (100 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was then purified by flash chromatography on silica gel using 3% MeOH in CH_2Cl_2 to give pure **19** as a yellowish solid (900 mg, 71%): ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.24 (s, 27H, 3×Si(CH_3)$_3$), 1.23-1.80 (m, 18H, 3×α-CH(CH_2)$_3$), 2.98-3.09 (m, 2H, CH_2NH), 3.16-3.28 (m, 4H, 2×CH_2NH), 3.59 (s, 3H, OCH_3), 4.20 (q, J = 7.8 Hz, 1H, α-CH), 4.33-4.40 (m, 2H, HC≡C, α-CH), 4.46 (q, J = 7.5 Hz, 1H, α-

CH), 7.49-7.55 (m, 8H, 4×H-3,5), 7.78-7.89 (m, 8H, 4×H-2,6), 7.95 (t, *J* = 5.3 Hz, 1H, CH_2NH), 8.32 (d, *J* = 7.3 Hz, 1H, α-NH), 8.44-8.55 (m, 4H, 2×CH_2NH, 2×α-NH); ^{13}C NMR (75.5 MHz, *d*$_6$-DMSO) δ -0.28 (9×CH_3, 3×$Si(CH_3)_3$), 22.56 (CH_2, α-$CHCH_2CH_2$), 23.10 (CH_2, α-$CHCH_2CH_2$), 23.17 (CH_2, α-$CHCH_2CH_2$), 28.50 (CH_2, CH_2CH_2NH), 28.64 (CH_2, CH_2CH_2NH), 28.74 (CH_2, CH_2CH_2NH), 30.31 (CH_2, α-$CHCH_2$), 31.15 (CH_2, α-$CHCH_2$), 31.28 (CH_2, α-$CHCH_2$), 38.11 (CH_2, CH_2NH), 39.02 (2×CH_2, 2×CH_2NH), 51.63 (OCH_3), 51.85 (α-CH), 53.11 (α-CH), 53.45 (α-CH), 82.63 (CH, C≡CH), 82.81 (C, C≡CH), 96.14 (2×C, 2×$CSi(CH_3)_3$), 96.26 (C, $CSi(CH_3)_3$), 104.37 (3×C, 3×C≡$CSi(CH_3)_3$), 124.31 (C), 124.48 (2×C), 124.71 (C), 127.31 (4×CH-2,6), 127.71 (4×CH-2,6), 131.28 (2×CH-3,5), 131.32 (4×CH-3,5), 131.35 (2×CH-3,5), 134.02 (C), 134.15 (C), 134.53 (2×C), 165.09 (NHC=O), 165.11 (NHC=O), 165.47 (NHC=O), 165.48 (NHC=O), 171.44 (C=O), 172.01 (C=O), 172.41 (C=O); HRMS (MALDI) 1167.4774 ($C_{64}H_{80}N_6NaO_8Si_3$ (MNa^+) requires 1167.5243).

Coumarin 343 labeled dendrimer (19a). DIPEA (7 μL, 0.04 mmol, 0.4 equiv) was added to a solution of compound **19** (114 mg, 0.10 mmol, 1.0 equiv), compound **13** (48 mg, 0.11 mmol, 1.1 equiv) and CuI (3.8 mg, 0.02 mmol, 0.2 equiv) in dry THF (2 mL). The resulting mixture was stirred overnight at room temperature. The solvent was then removed under vacuum and the residue was passed through a short silica gel column using 4 % MeOH in CH_2Cl_2 as eluent to afford pure **19a** as a yellow solid (146 mg, 92 %): ^{1}H NMR (600 MHz, *d*$_6$-DMSO) δ 0.23-0.24 (m, 27H, 3×$Si(CH_3)_3$), 1.27-1.48 (m, 8H, 3×α-$CHCH_2CH_2$, α-$CHCH_2$), 1.48-1.64 (m, 5H 2×α-$CHCH_2$, α-$CH(CH_2)_2CHH$), 1.67-1.80 (m, 5H, 2×α-$CH(CH_2)_2CH_2$, α-$CH(CH_2)_2CHH$), 1.80-1.86 (m, 4H, 2×NCH_2CH_2), 2.66-2.69 (m, 4H, 2×$N(CH_2)_2CH_2$), 3.00-3.10 (m, 2H, α-$CH(CH_2)_3CH_2$), 3.19-3.31 (m, 8H, 2×α-$CH(CH_2)_3CH_2$, 2×NCH_2), 3.44 (m, 2H, OCH_2CH_2NH), 3.51 (t, *J* = 5.4 Hz, 2H, OCH_2), 3.54-3.55 (m, 2H, OCH_2), 3.59 (m, 5H, OCH_3, OCH_2), 3.90 (t, *J*

= 5.2 Hz, 2H, triazole-$CH_2C\mathit{H}_2$), 4.21 (q, *J* = 7.7 Hz, 1H, α-CH), 4.39 (q, *J* = 8 Hz, 1H, α-CH), 4.47 (q, *J* = 8.2 Hz, 1H, α-CH), 4.59 (t, *J* = 5.1 Hz, 2H, triazole-$C\mathit{H}_2$), 7.19 (s, 1H, CH-vinyl(coumarin)), 7.49-7.52 (m, 6H, 3×H-3,5), 7.80-7.81 (m, 4H, 2×H-2,6), 7.86-7.90 (m, 4H, H-2,6, H-3,5), 7.94-7.98 (m, 3H, H-2,6, $CH_2N\mathit{H}$), 8.34 (d, *J* = 7.2 Hz, 1H, α-NH), 8.39 (d, *J* = 7.8 Hz, 1H, α-NH), 8.48-8.49 (m, 2H, CH-aryl(coumarin), α-NH), 8.53-8.54 (m, 2H, 2×$CH_2N\mathit{H}$), 8.61 (s, 1H, H(triazole)), 8.80 (t, *J* = 7.8 Hz, 1H, $CH_2N\mathit{H}$); ^{13}C NMR (150 MHz, d_6-DMSO) δ -0.29 (3×CH_3, $Si(CH_3)_3$), -0.27 (6×CH_3, 2×$Si(CH_3)_3$), 19.47 (2×CH_2, $NCH_2CH_2\mathit{C}H_2$, $NCH_2\mathit{C}H_2$), 20.43 (CH_2, $NCH_2\mathit{C}H_2$), 22.60 (CH_2, α-$CHCH_2\mathit{C}H_2$), 23.13 (CH_2, α-$CHCH_2\mathit{C}H_2$), 23.25 (CH_2, α-$CHCH_2\mathit{C}H_2$), 26.69 (CH_2, $NCH_2CH_2\mathit{C}H_2$), 28.54 (CH_2, α-$CH(CH_2)_2\mathit{C}H_2$), 28.73 (CH_2, α-$CH(CH_2)_2\mathit{C}H_2$), 28.75 (CH_2, α-$CH(CH_2)_2\mathit{C}H_2$), 30.34 (CH_2, α-$CH\mathit{C}H_2$), 31.17 (CH_2, α-$CH\mathit{C}H_2$), 31.44 (CH_2, α-$CH\mathit{C}H_2$), 38.15 (CH_2, α-$CH(CH_2)_3\mathit{C}H_2$), 38.68 (CH_2, $NH\mathit{C}H_2CH_2O$), 39.15 (2×CH_2, 2×α-$CH(CH_2)_3\mathit{C}H_2$), 48.88 (CH_2, NCH_2), 49.42 (CH_2, NCH_2), 49.61 (triazole-CH_2), 51.65 (OCH_3), 51.88 (α-CH), 53.12 (α-CH), 53.42 (α-CH), 68.59 (CH_2O), 68.99 (CH_2O), 69.47 (CH_2O), 69.56 (CH_2O), 96.16 (2×C, 2×$\mathit{C}Si(CH_3)_3$), 96.28 (C, $\mathit{C}Si(CH_3)_3$), 104.38 (3×C, 2×$\mathit{C}{\equiv}CSi(CH_3)_3$, C), 104.48 (C, $\mathit{C}{\equiv}CSi(CH_3)_3$), 107.28 (C), 107.63 (C), 119.29 (C), 122.38 (CH(triazole)), 124.50 (2×C, 2×CH-aryl), 124.72 (C), 127.03 (CH-vinyl), 127.31 (2×CH-aryl), 127.32 (2×CH-aryl), 127.71 (2×CH-aryl), 128.11 (2×CH-aryl), 131.29 (2×CH-aryl), 131.34 (4×CH-aryl), 133.05 (C), 133.33 (C), 134.02 (C), 134.52 (2×C), 145.38 (C), 147.45 (CH-aryl), 147.86 (C), 151.96 (C), 161.81 (C=O), 162.38 (C=O), 165.09 (NHC=O), 165.12 (NHC=O), 165.47 (NHC=O), 165.80 (NHC=O), 171.60 (C=O), 172.05 (C=O), 172.44 (C=O); HRMS (MALDI) 1609.1865 ($C_{86}H_{107}N_{11}NaO_{13}Si_3$ (MNa^+) requires 1608.7255).

Trimannose fluorescent probe (20). A solution of compound **19a** (22 mg, 0.014 mmol, 1 equiv), 3-azidopropyl α-D-mannopyranoside **12** (22 mg, 0.083 mmol, 6 equiv) and CuF_2 (8

mg, 0.083 mmol, 6 equiv) in MeOH (2 mL) was stirred for 40 h at 45 °C (The reaction was monitored by MALDI MS). The crude mixture was then purified by RP-HPLC (Zorbax Eclipse C18, linear gradient 0-100% CH_3CN in water over 40 min, *t*= 29.4 min) and lyophilization of the appropriate fractions afforded pure **20** (24 mg, 80%): ^{1}H NMR (600 MHz, d_6-DMSO+D_2O) δ 1.27-1.46 (m, 8H, 3×α-$CHCH_2CH_2$, α-$CHCH_2$), 1.49-1.65 (m, 5H, 2×α-$CHCH_2$, α-$CH(CH_2)_2CH$), 1.67-1.81 (m, 9H, 2×α-$CH(CH_2)_2CH_2$, α-$CH(CH_2)_2CH$H, 2×NCH_2CH_2), 2.09-2.16 (m, 6H, 3×$OCH_2CH_2CH_2$), 2.63-2.66 (m, 4H, 2×$N(CH_2)_2CH_2$), 3.01-3.09 (m, 2H, α-$CH(CH_2)_3CH_2$), 3.20-3.66 (m, 43H, 2×α-$CH(CH_2)_3CH_2$, 2×NCH_2, $O(CH_2)_2O(CH_2)_2NH$, OCH_3, 6×H-6, 3×H-2, 3×H-3, 3×H-4, 3×H-5, 3×$OCH_2(CH_2)_2$), 3.88 (t, *J* = 5.2 Hz, 2H, triazole-CH_2CH_2O), 4.21 (m, 1H, α-CH), 4.38 (t, *J* = 7.3 Hz, 1H, α-CH), 4.42-4.51 (m, 7H, α-CH, 3×$O(CH_2)_2CH_2$-triazole), 4.56-4.61 (m, 5H, OCH_2CH_2-triazole, 3×H-1), 7.16 (s, 1H, CH-vinyl(coumarin)), 7.83-7.93 (m, 16H, 16×CH-aryl), 8.37 (d, *J* =7.1 Hz, 1H, α-NH), 8.45 (s, 1H, CH-aryl(coumarin)), 8.54 (s, 1H, H(triazole)), 8.62-8.63 (m, 3H, 3×H(triazole)); ^{13}C NMR (150 MHz, d_6-DMSO+D_2O) δ 19.49 (2×CH_2, $N(CH_2)_2CH_2$, NCH_2CH_2), 20.45 (CH_2, NCH_2CH_2), 22.65 (CH_2, α-$CHCH_2CH_2$), 23.19 (CH_2, α-$CHCH_2CH_2$), 23.25 (CH_2, α-$CHCH_2CH_2$), 26.72 (CH_2, $N(CH_2)_2CH_2$), 28.60 (CH_2, α-$CH(CH_2)_2CH_2$), 28.79 (CH_2, α-$CH(CH_2)_2CH_2$), 28.88 (CH_2, α-$CH(CH_2)_2CH_2$), 29.73 (3×CH_2, 3×$OCH_2CH_2CH_2$), 30.40 (CH_2, α-$CHCH_2$), 31.33 (CH_2, α-$CHCH_2$), 31.44 (CH_2, α-$CHCH_2$), 38.21 (CH_2, α-$CH(CH_2)_3CH_2$), 38.71 (CH_2, $NHCH_2CH_2O$), 39.16 (2×CH_2, 2×α-$CH(CH_2)_3CH_2$), 47.00 (3×CH_2, 3×$O(CH_2)_2CH_2$), 48.91 (CH_2, NCH_2), 49.45 (CH_2, NCH_2), 49.64 (CH_2-triazole), 51.71 (OCH_3), 51.95 (α-CH), 53.12 (α-CH), 53.49 (α-CH), 61.16 (3×CH_2-6), 63.20 (3×CH_2, 3×$OCH_2(CH_2)_2$), 66.91 (3×CH-4), 68.61 (CH_2O), 69.00 (CH_2O), 69.49 (CH_2O), 69.59 (CH_2O), 70.21 (3×CH-2), 70.95 (3×CH-3), 74.07 (3×CH-5), 99.92 (3×CH-1), 104.52 (C), 107.30 (C), 107.61 (C), 119.35 (C), 122.10 (CH(triazole)), 122.11

(CH(triazole)), 122.18 (CH(triazole)), 122.44 (CH(triazole)), 124.55 (2×CH-aryl), 124.65 (2×CH-aryl), 124.70 (4×CH-aryl), 127.06 (CH-vinyl(coumarin)), 127.76 (4×CH-aryl), 128.16 (4×CH-aryl), 133.12 (2×C), 133.17 (2×C), 133.33 (C), 133.41 (C), 133.65 (2×C), 145.41 (C), 145.52 (C), 145.55 (2×C), 147.48 (CH-aryl), 147.92 (C), 151.99 (C), 161.86 (C=O), 162.44 (C=O), 165.63 (2×NHC=O), 165.89 (NHC=O), 165.93 (NHC=O), 171.69 (C=O), 172.25 (C=O), 172.51 (C=O); HRMS (MALDI) 2182.6877 ($C_{104}H_{134}N_{20}NaO_{31}$ (MNa^+) requires 2181.9422).

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CHAPTER 3

CONVERGENT ASSEMBLY AND SURFACE MODIFICATION OF MULTI-FUNCTIONAL DENDRIMERS BY THREE CONSEQUTIVE "CLICK" REACTIONS[†]

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Abstract

Multi-functional dendrimers bearing two or more surface functionalities have the promise to provide smart drug delivery devices that can, for example, combine tissue targeting and imaging or be directed more precisely to a specific tissue or cell type. We have developed a concise synthetic methodology for efficient dendrimer assembly and hetero-bi-functionalization based on three sequential azide-alkyne cycloadditions. The methodology is compatible with biologically important compounds rich in chemical functionalities such as peptides, carbohydrates, and fluorescent tags. In the approach, a strain promoted azide-alkyne cycloaddition (SPAAC) between polyester dendrons modified at the focal point with an azido and 4-dibenzocyclooctynol (DIBO) moiety provided dendrimers bearing terminal and TMS-protected alkynes at the periphery. The terminal alkynes were outfitted with azido-modified polyethylene glycol (PEG) chains or galactosyl residues using Cu(I) catalyzed azide-alkyne cycloadditions (CuAAC). Next, a one-pot TMS-deprotection and second "click" reaction of the resulting terminal alkyne with azido-containing compounds gave multi-functional dendrimers bearing complex biologically active moieties at the periphery.

Introduction

Dendrimers are emerging as promising materials for the development of imaging devices and drug and gene delivery vehicles.[1] Attractive properties of dendrimers include chemical homogeneity, tunability of biodistribution and pharmacokinetics by regulating size and controlled degradation by judicious choice of dendrimer chemistry.[2] Furthermore, the typical architecture of dendrimers results in the formation of cavities, which can entrap pharmaceutically active substances.[3] Moreover, the surface of dendrimers can be modified by prodrugs, imaging modules such as fluorescent tags, CT and MRI contrast agents,[4] polyethylene glycol to increase water solubility and improve biocompatibility,[5] and by cell tissue targeting ligands such as folic acid or RGD peptides to increase therapeutic efficiency.[6] Surface modification of dendrimers with a targeting device benefits from high multivalent densities, which will strengthen ligand-receptor binding as a result of a cluster effect.[7] A particularly attractive approach for surface modification of dendrimers is a Cu(I) catalyzed 1,3-dipolar cycloaddition of azides with terminal alkynes (CuAAC) to give stable 1,2,3-triazoles.[8] CuAAC combines exceptional chemoselectivity with a lack of byproducts and high yields. It has been used to efficiently derivatize dendrimers with unprotected peptides,[9] carbohydrates,[10] and other complex compounds.[11]

It is to be expected that dendrimers modified by several different peripheral entities can combine functions such as tissue targeting and imaging or be directed more precisely to a specific tissue or cell type.[12] Usually, multi-functional dendrimers are prepared by a random chemical coupling reaction, which unfortunately leads to unwanted dispersity.[13] A more attractive approach uses dendritic molecules or polymers having two or more orthogonal functionalities or protecting groups.[14] In particular, azides or alkynes for CuAAC combined with hydroxyls for etherification[15] or aldehydes for hydrazone formation[16] have been successfully

employed as sets of the orthogonal functionalities. Dendrimers have also been multi-functionalized using CuAAC in a sequential manner. In this approach, polyester dendrimers modified by mannoside-targeting moieties and coumarin fluorescent tags were prepared by starting with a dendrimer having peripheral alcohols and isopropylidene acetals.[17] The alcohols of the dendrimer could be modified by terminal alkynes, which could then be coupled with azide-modified coumarin. Removal of isopropylidene acetals gave alcohols and a repetition of alkyne formation and CuAAC led to the controlled introduction of peripheral mannosides. More recently, bi-functional[18] and tri-functional[19] dendrimers were constructed by "click" reaction followed by coupling of azide bearing dendron to the dendrimer core, thus enabling a surface modification by a second CuAAC. Despite many attractive features of these methods, the limited chemoselectivity of conventional functional groups such as alcohol, amine, carboxylic acids and carbonyls and in some cases the relatively large number of chemical steps for orthogonal group installation, places restrictions on the type of functionality that can be attached to a dendritic surface. We report here a versatile approach for selective surface modification of dendrimers by a strain-promoted alkyne-azide cycloaddition (SPAAC)[20] between two dendrons modified by a focal dibenzocyclooctyne or azide and having peripheral alkynes or TMS-protected alkynes, respectively. This ligation exploits a selective reaction of a strained alkyne with an azide in the presence of terminal alkynes.[21] The terminal alkynes can, however, be selectively modified with an azide-containing moiety using a Cu(I) catalyst. In a third step, a second type of surface functionality can be installed in a controlled manner by removal of the TMS-protecting groups followed by another CuAAC (Figure 3.1).

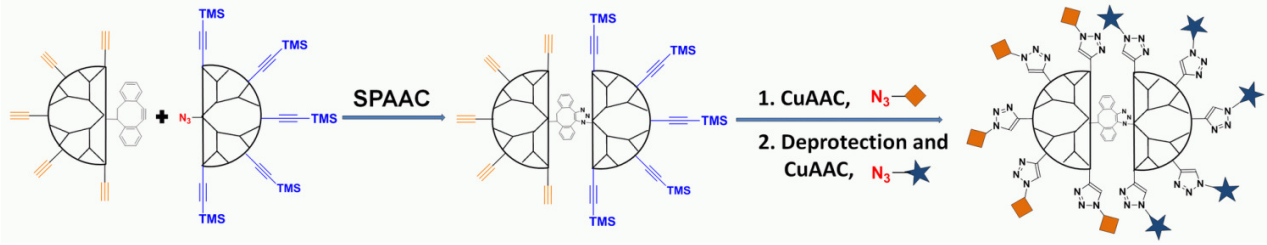


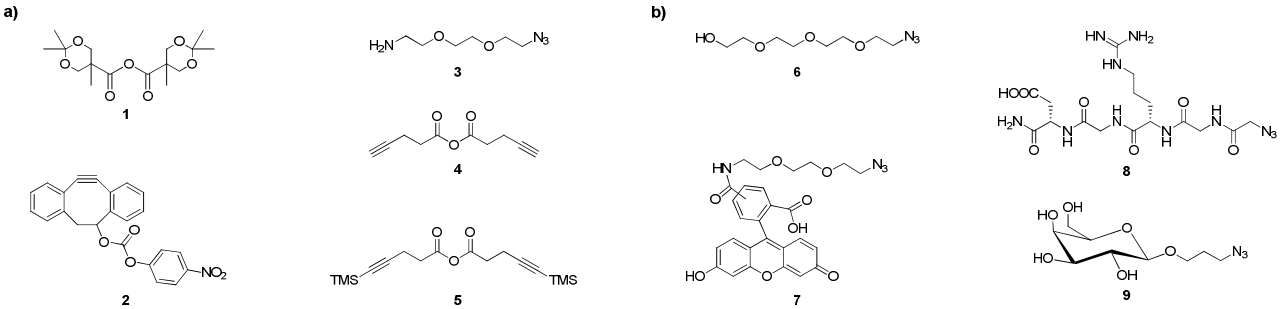
Figure 3.1 General concept of multi-functional dendrimer synthesis by three consecutive "click" reactions

The excellent chemoselectivity of SPAAC and CuAAC ensures that a wide variety of functionalities, such as biological relevant carbohydrates and peptides, can be attached in a controlled manner to the periphery of dendrimers.

Results and Discussion

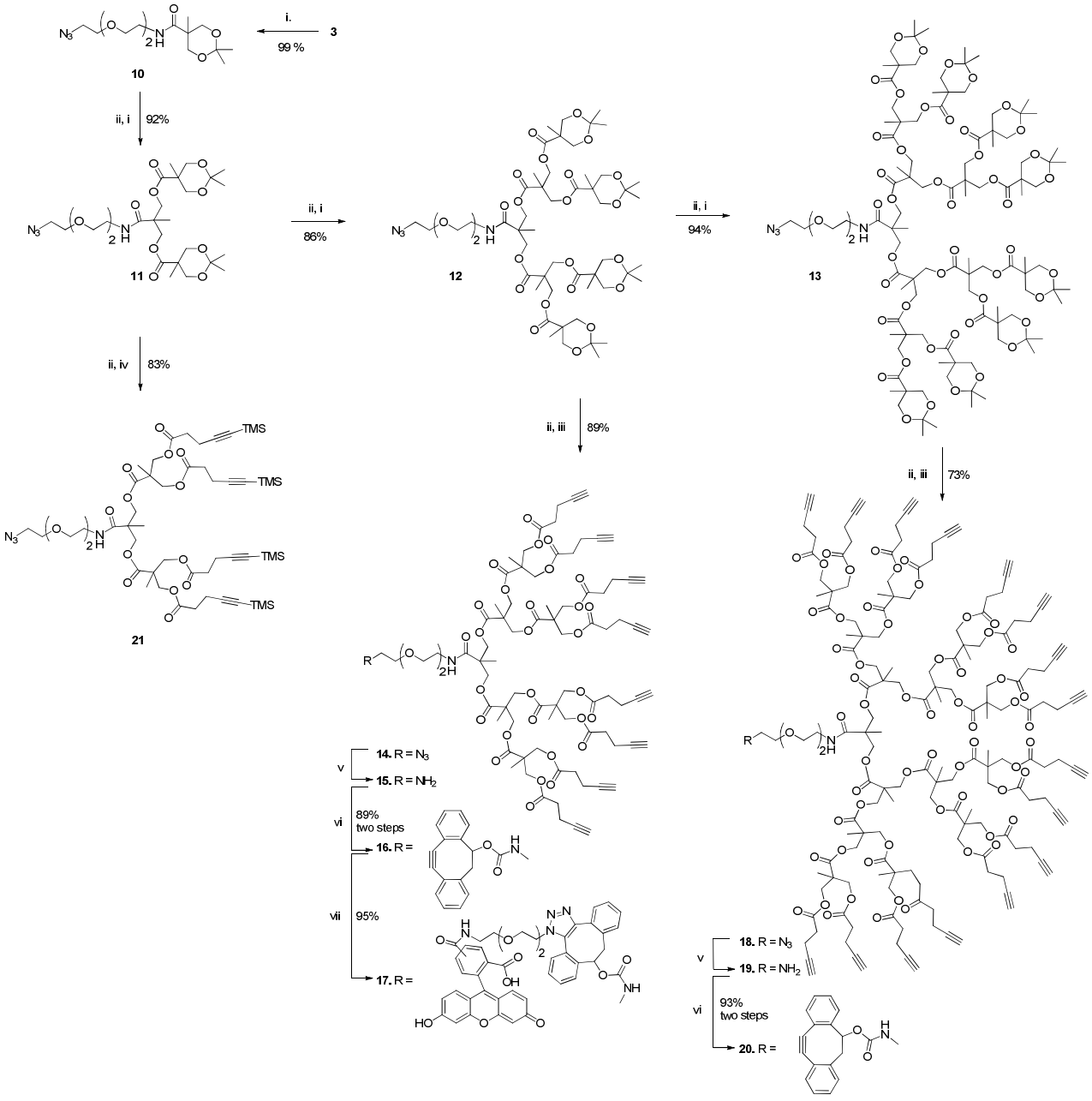
First, we examined whether a strain-promoted alkyne-azide cycloaddition can be utilized for the ligation of two dendrons. Such a reaction is challenging due to steric hindrance at dendron focal points, which may render couplings inefficient leading to low yields and loss of expensive dendrons. Thus, we synthesized generation three- and four-dendrons **14**, **16**, **18** and **20** having a polyester dendritic framework based on 2,2-bis(hydroxymethyl) propionic acid (bis-MPA)[22] (Scheme 3.1) and bearing an azide or a 4-dibenzocyclooctynol (DIBO) moiety[20c] at the focal point (Scheme 3.2).

Scheme 3.1 a) Dendron building blocks. b) Azides for dendrimer derivatization



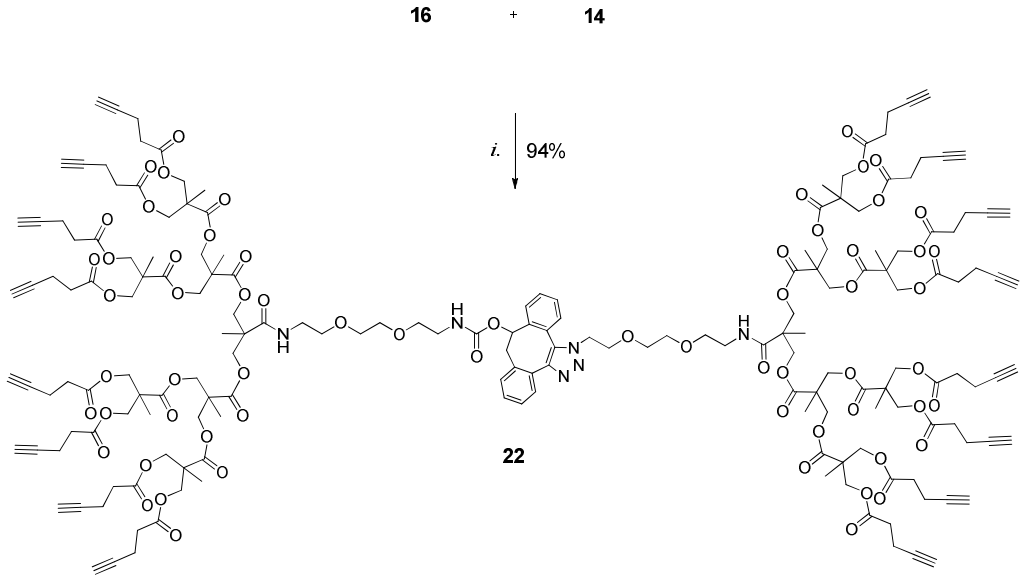
A polyester framework was selected because of its intrinsic biodegradability[23] and good solubility in organic solvents.[24] DIBO was used because it reacts fast with azido-containing compounds in the absence of a metal catalyst, can be prepared by a simple synthetic approach, is nontoxic, and can easily be attached to a variety of probes. Dendron synthesis started with a coupling of 2-(2-(2-azidoethoxy)ethoxy)ethanamine[25] (**3**) (Scheme 3.1) with isopropylidene protected bis-MPA anhydride **1**[22] in the presence of pyridine and dimethyaminopyridine (DMAP) in DCM to give amide **10**, which was treated with Dowex H^+ resin in MeOH to remove the isopropylidene acetals and reveal alcohols. Each subsequent generation was introduced by reaction of hydroxyls with anhydride **1** followed by removal of the isopropylidene protecting groups. In this way, polyester dendrons **11**, **12** and **13** were synthesized having masked alcohols at the periphery and an azide at the focal point. After deprotection of the isopropylidene acetals of **12** and **13**, peripheral alkynes were introduced by treatment with pent-4-ynoic anhydride (**4**) to give **14** and **18**, respectively. Alternatively, treatment of **11** with Dowex-H^+ followed by reaction of the resulting alcohols with 5-(trimethylsilyl)pent-4-ynoic anhydride (**5**) gave **21**, which has alkynes protected by trimethylsilyl (TMS) groups. All transformation proceeded in high yield leaving the important azide moiety at the focal point of the dendrons intact. The azido-containing dendrons **14** and **18** were the starting material for the preparation of the DIBO containing derivatives **16** and **20**, respectively. Thus, reduction of the azides of **14** and **18** with trimethylphosphine in a mixture of THF and water gave the corresponding amines **15** and **19**, which were immediately treated with the activated carbonate of DIBO (**2**)[20c] to provide the requisite compounds **16** and **20** in yields of 89 and 93%, respectively. Having azide- and DIBO-modified dendrons at hand, attention was focused on SPAAC-mediated ligation[26] of these derivatives.

Scheme 3.2 Synthesis of dendrons, introduction of dibenzocyclooctyne and copper free "click" reaction. Reaction conditions: *i.* isopropylidene-2,2-bis(hydroxymethyl)propionic anhydride (**1**), DMAP, Py, DCM, 0˚C then RT, 12-18 h. *ii.* DOWEX® H^+ resin, MeOH, 50˚C, 2-24 h. *iii.* pent-4-ynoic anhydride (**4**), DMAP, Py, DCM, 0˚C then RT, 18 h. *iv.* 5-(trimethylsilyl)pent-4-ynoic anhydride (**5**), DMAP, Py, DCM, 0˚C then RT, 18 h. *v.* PMe_3 10 eq., THF:H_2O, 9:1 v/v, 3 h; *vi.* **2**, DMF, DIPEA, 48 h; *vii.* **7**, THF, 2 h



First a symmetrical G3-G3 dendrimer **22** with alkynes on periphery was constricted by coupling DIBO and azide containing dendrons **16** and **14** (Scheme 3.3). As determined from TLC the reaction was over in 11h and led to almost quantitative formation of the product, after simple chromatography purification **22** was isolated in 94 % yield. We then investigated whether or not SPAAC will be equally efficient in coupling of higher generation dendrons **18** and **20**.

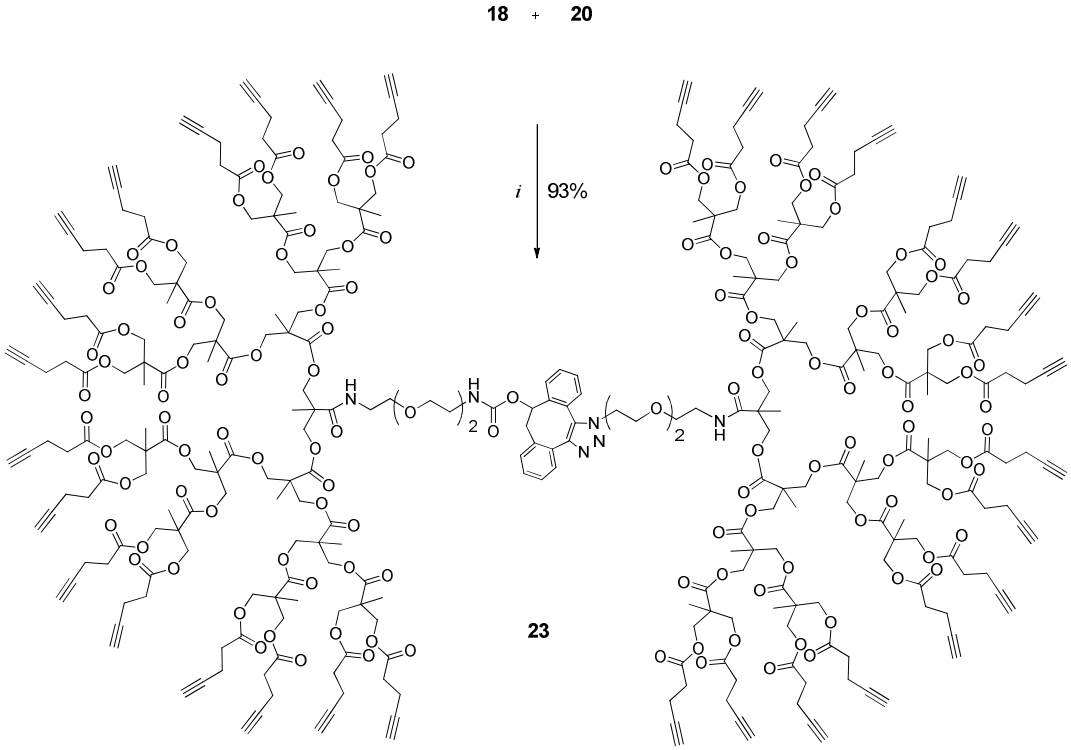
Scheme 3.3 Synthesis of dendrimer **22**. Reaction conditions: *i.* THF, 11 h



Gratifyingly, reaction of the G4 dendrons **18** and **20** in THF at room temperature proceeded smoothly and gave, after a reaction time of 24 h, symmetrical dendrimer **23** in a yield of 93% (Scheme 3.4). In addition, the focal DIBO moiety of **16** could be employed for installing a fluorescent probe and reaction with azido-modified fluorescein **7** gave derivative **17** (Scheme 3.2). Importantly, the copper free coupling required only a stoichiometric quantity of dendrons. In the presence of a catalytic amount of $CuSO_4$, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) and sodium ascorbate in a mixture of THF and water, the alkynes of **17, 22** and **23** could be reacted with azido-containing compounds. Thus, a galactose-modified

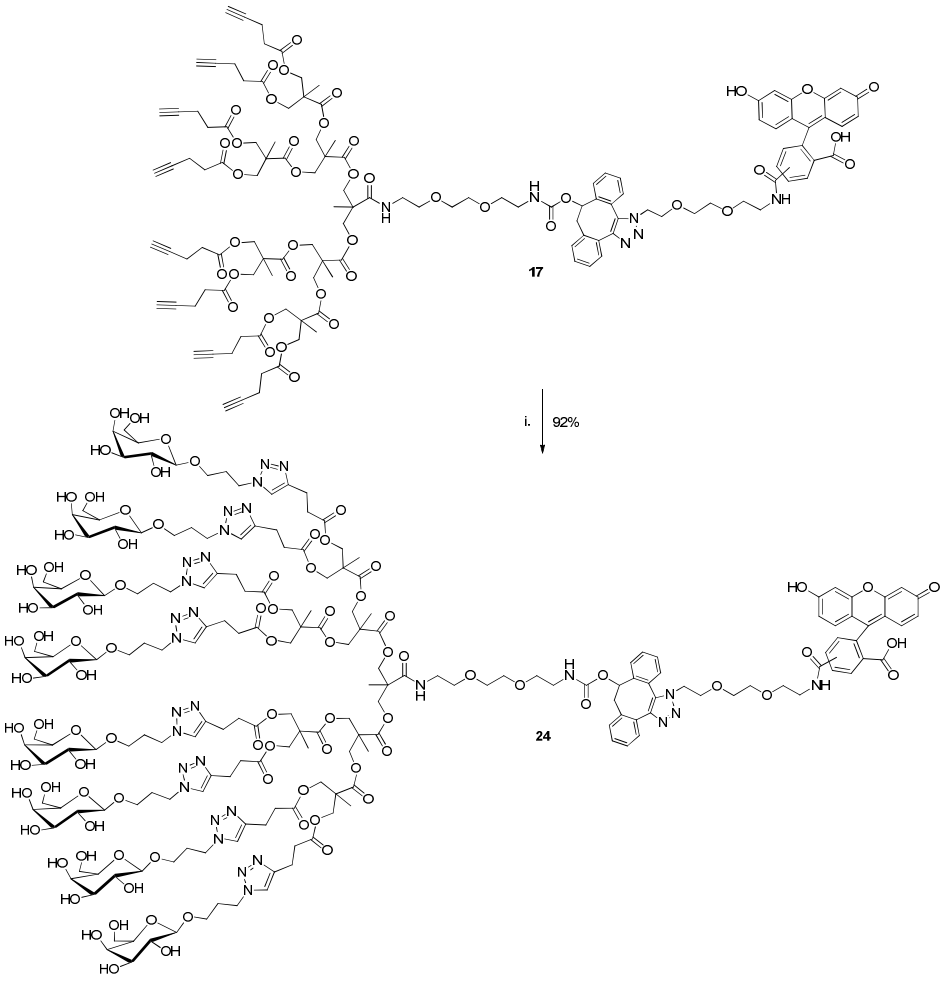
fluorescently labeled dendron **24** was prepared in 92% yield by reacting dendron **17** with 3-azidopropyl β-D-galactopyranoside (**9**) (Scheme 3.5).

Scheme 3.4 Dendrimer assembly *via* SPAAC. Reaction conditions: *i*. THF, 24 h



Similarly, a symmetrical G3-G3 dendron **22** with terminal alkynes on periphery was derivatized with unprotected galactose derivatives **9** using $CuSO_4$, Na ascorbate and TBTA in THF:H_2O mixture to give a glycodendron **25** in 90% yield (Scheme 3.6). We then investigated if CuAAC could be used to efficiently derivatize all 32 alkyne groups on the periphery of a G4-G4 dendron **23** (Scheme 3.7). Gratifyingly, peripheral modified dendrimers **26** and **27** were obtained in good yields by reaction of **23** with 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol[27] (**6**) and azido-containing galactoside[28] **9**, respectively (Scheme 3.7).

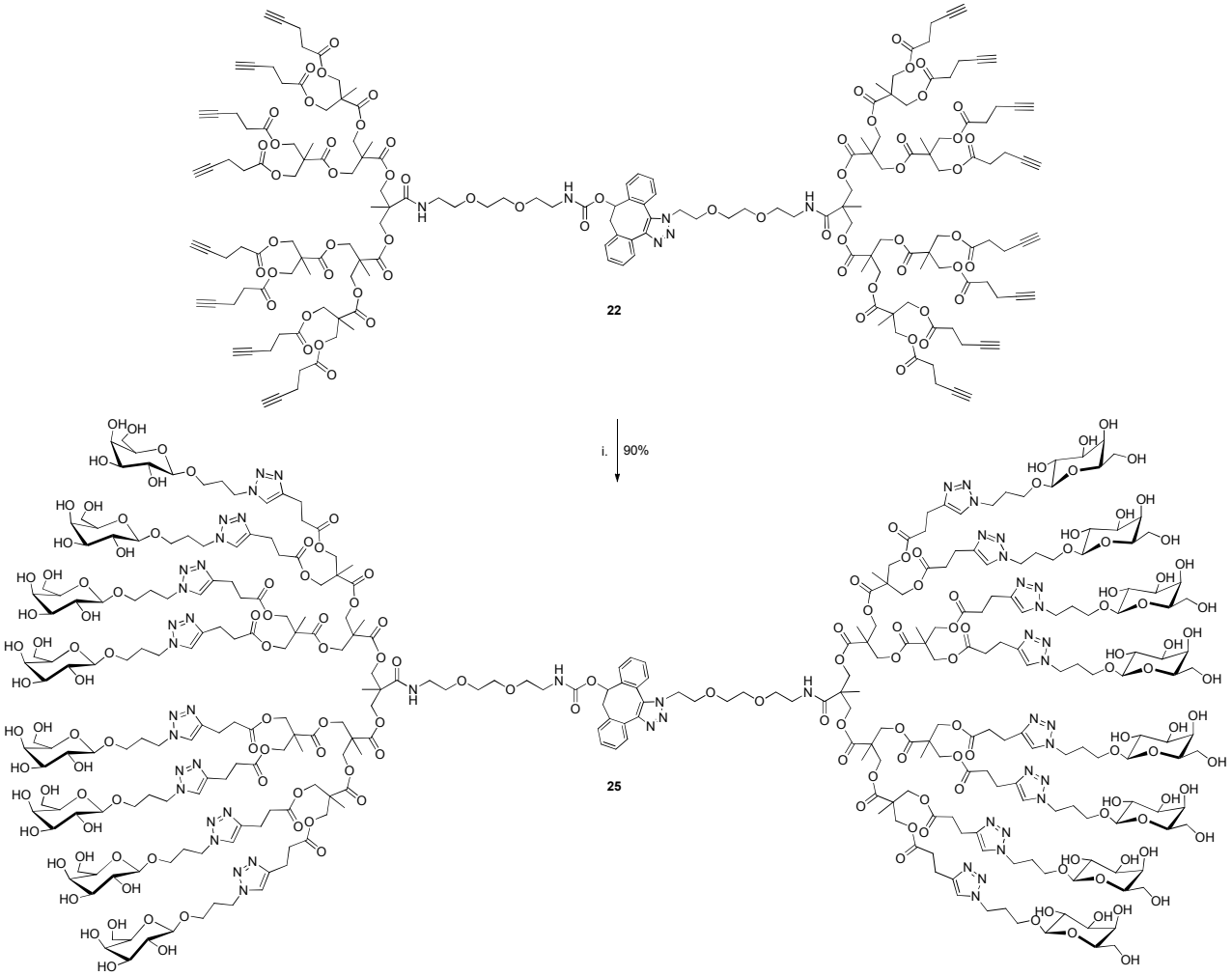
Scheme 3.5 Synthesis of glycodendrimer **24**. Reaction conditions: *i.* **9**, $CuSO_4$, Na ascorbate, TBTA, THF:H_2O, 18 h



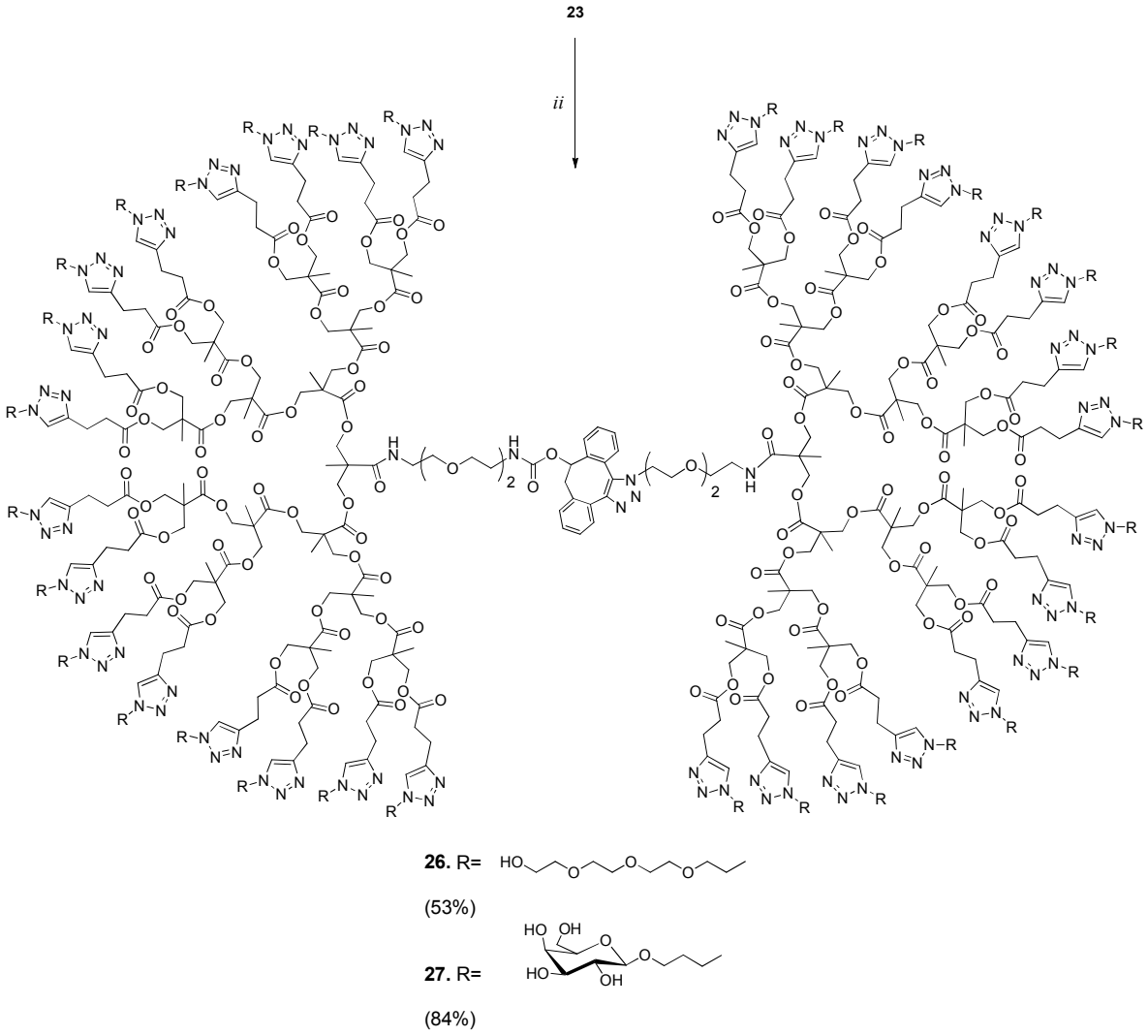
The ^{1}H-NMR spectra of the CuAAC products showed characteristic triazole signals (8 ppm), integration of which along with the unique CH_2–triazole signals (4.5 ppm) of the galactosyl or tetraethyleneglycol residues, yielded in each case thirty-two triazole residues per dendrimer molecule (Figure 3.2). Complete surface derivatization of compound **27** was additionally confirmed by quantitative sugar analysis (31.8±0.2 galactosyl residues). Next, we explored whether SPAAC can facilitate coupling of two dendrons having either terminal or TMS-protected alkynes to give dendrimers that can be modified in a controlled manner by two different surface entities. In this respect, a number of studies have shown that CuAAC can be

performed without affecting a TMS-protected alkyne.[29] However, the TMS protecting group can easily be removed by reagents such as tetrabutylammonium fluoride (TBAF) or silver salts and the resulting terminal alkyne used in a subsequent "click" reaction. The challenge for using this methodology for dendrimer modification is that multiple TMS-protected alkynes have to stay intact during dendrimer assembly and the first CuAAC.

Scheme 3.6 Synthesis of glycodendrimer **25**. Reaction conditions: *i.* **9**, $CuSO_4$, Na ascorbate, TBTA, THF:H_2O, 18 h



Scheme 3.7 CuAAC-mediated derivatization of dendrimer **23**. Reaction conditions: *ii*. **6** or **9**, $CuSO_4$, Na ascorbate, TBTA, THF:H_2O, 18 h



Thus, SPAAC mediated coupling of **16** with **21** in THF for 5 h gave clean formation of the asymmetrical dendrimer **28**, which has terminal and TMS-protected alkynes at its periphery (Scheme 3.8). Next, **28** was subjected to azido-containing tetraethyleneglycol **6** in the presence of a catalytic amount of CuI and *N,N*-diisopropylethylamine (DIPEA) (Scheme 3.9).

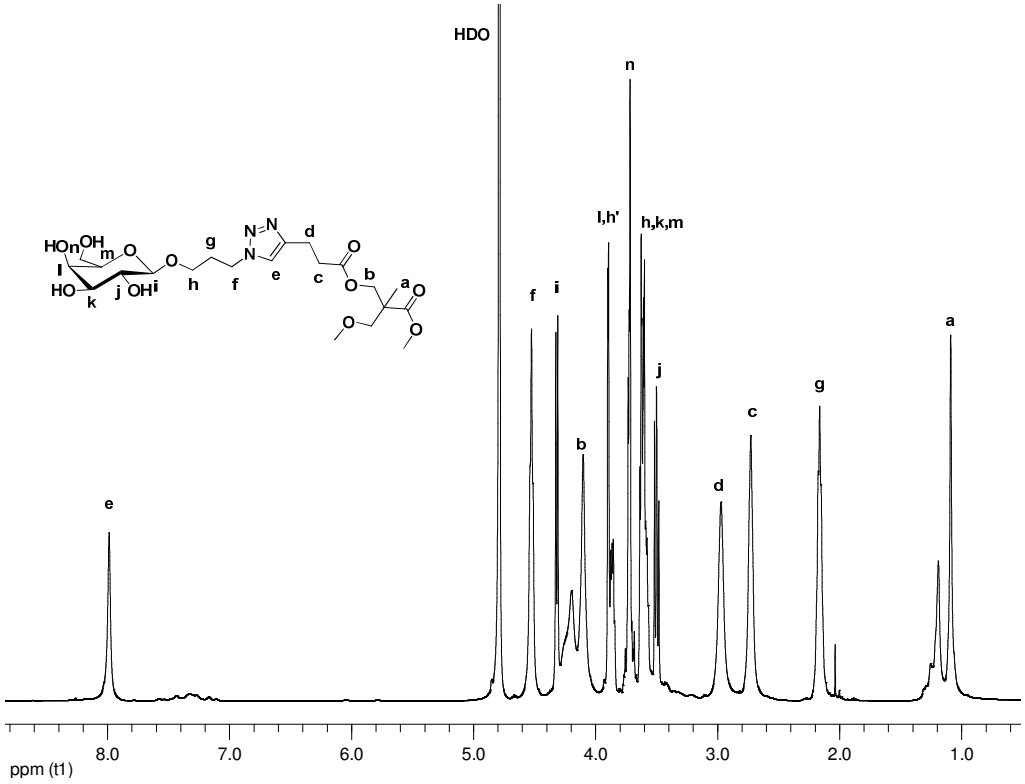
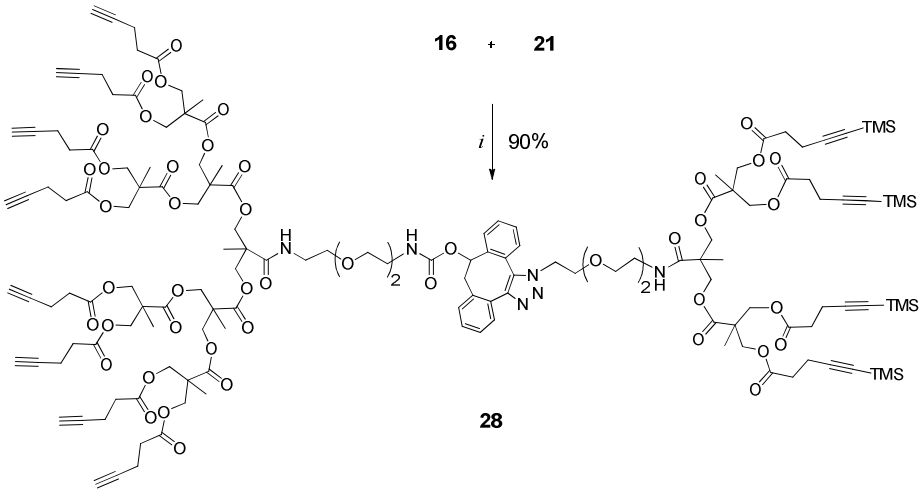
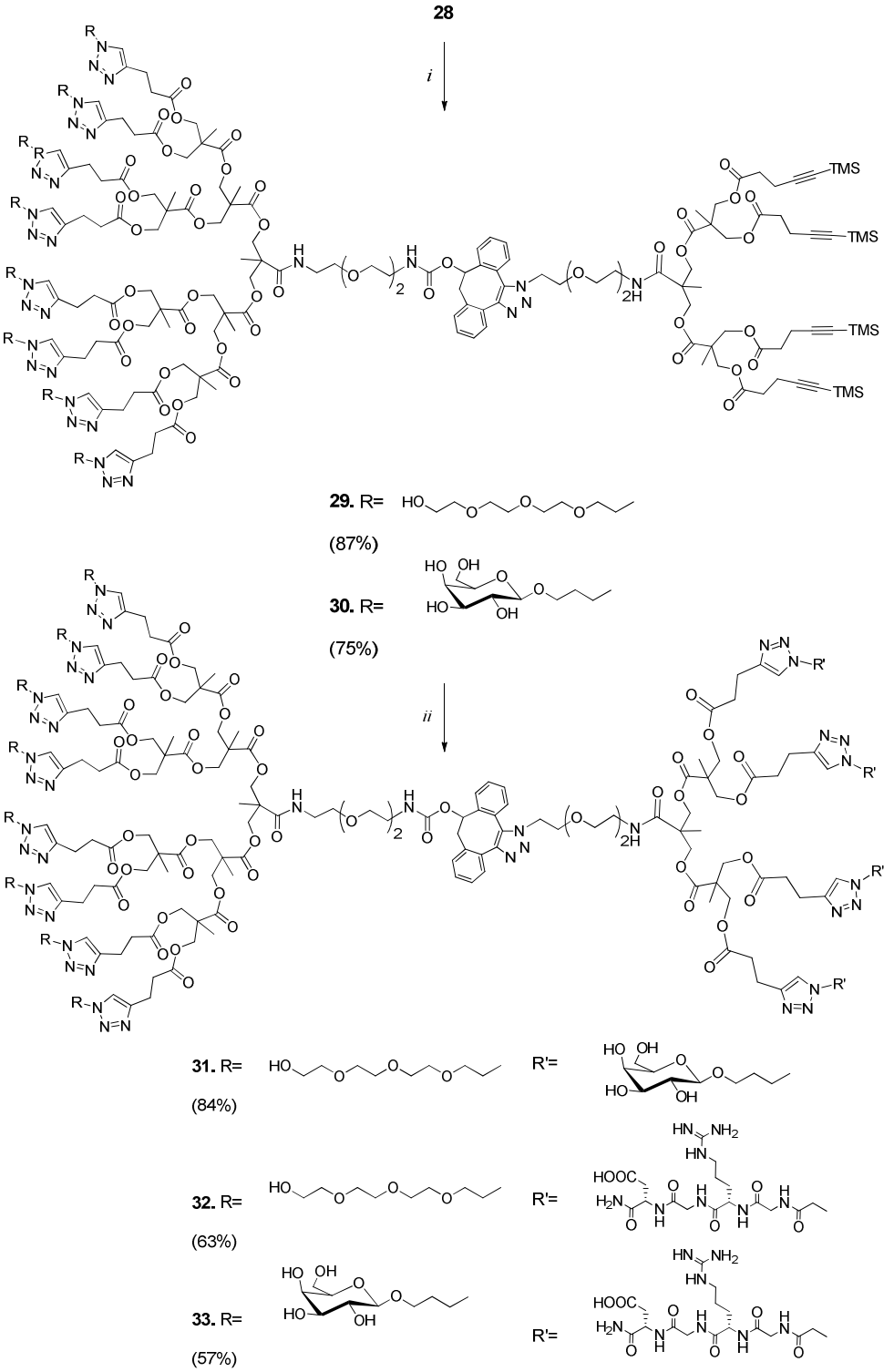


Figure 3.2 ^{1}H-NMR spectrum of glycodendrimer **27**. (D_2O, 500 MHz)

Scheme 3.8 SPAAC reaction leading to bi-functional dendrimer **28**. Reaction conditions: *i.* THF, 5 h



Scheme 3.9 Bi-functionalization of dendrimer **28**. Reaction conditions: *i*. **6**, CuI, DIPEA, THF, 4 h; *ii*. **8** or **9**, CuF_2, MeOH or MeOH:H_2O, 40°C, 8-40 h



Under these conditions, the terminal alkynes underwent a clean cycloaddition and after a reaction time of 4 h and purification by LH-20 size exclusion column chromatography, dendrimer **29** was obtained in a yield of 87%. Similarly, a reaction between asymmetrical dendrimer **28** and unprotected galactoside **9** afforded glycodendrimer **30** in a yield of 75%. Careful analysis of the structures of **28**, **29** and **30** by ^{1}H-NMR and MALDI ToF revealed that the TMS-protected alkynes had remained intact during the SPAAC and CuAAC "click" reactions. Partial desilylation was, however, observed when a combination of $CuSO_4$ and sodium ascorbate was used for the CuAAC. Previously, we found that CuF_2 can efficiently unmask TMS-modified alkynes and promote cycloadditions with azides.[30] Fortunately, this protocol could be employed for the modification of **29** and reactions with RGD peptide **8** and galactoside **9** proceeded smoothly when methanol was used as a solvent at a temperature of 40°C, to give bi-functional dendrimers **31** and **32**, respectively. Bi-functional dendrimer **33** bearing unprotected galactoside residues and RGD peptides was obtained in a similar manner by treatment of glycodendrimer **30** with peptide **8** with CuF_2 in methanol-water mixture at 40˚C. The absence of characteristic TMS proton signals in the ^{1}H-NMR and correct integral areas of sugar and peptide protons indicated complete derivatization of dendrimers **29** and **30**.

Conclusion

We have developed a convenient approach for dendrimer assembly and peripheral functionalization using three consecutive azide-alkyne cycloadditions. Strain promoted azide-alkyne cycloaddition was established as an effective and chemoselective method for coupling of dendrons to give symmetrical and asymmetrical dendrimers bearing alkynes on the periphery. Differentiated terminal and TMS-protected peripheral alkynes were efficiently modified with different combinations of model PEG, galactosyl, and peptide-azides, bearing no protecting

groups. The methodology is compatible with compounds that are rich in chemical functionalities such as peptides, carbohydrates, and fluorescent tags. Furthermore, only three consecutive steps are required for dendron coupling and installment of two-different surface entities. Recently, photo-,[31] thiol-ene,[32] and strain-promoted alkyne-nitrone[33] "click" reactions have been introduced, which also display excellent chemoselectivity and it is to be expected that integration of these reactions in the approach reported here will give easy access to even more complex dendritic structures.

Experimental Section

All chemicals were purchased from Sigma-Aldrich unless stated otherwise. L-amino acid derivatives and resins were purchased from NovaBioChem and Biosystems. Methylene chloride (DCM) was dried over calcium hydride. Anhydrous tetrahydrofuran (THF), methanol, and *N,N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich and EMD, respectively. All esterification, amidation, Staudinger reduction, $CuSO_4$ and CuI mediated reactions were carried out under an argon atmosphere. Reactions were performed at room temperature (20-22 °C), unless stated otherwise. Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed silica gel 60 (F254) plates, visualized using UV254nm and potassium permanganate and cerium molybdate dips as appropriate. Flash chromatography was carried out using silica gel G60 (SiliCycle, 60-200μm 60 Å) as the stationary phase. Solid-Phase Peptide Synthesis (SPPS) was performed on a Applied Biosystems, ABI 433A peptide synthesizer equipped with UV-detector using L-N^{α}-Fmoc-protected amino acids and 2-(1H-benzotriazole-1-yl)-oxy-1,1,3,3-tertamethyl hexafluorophosphate (HBTU) / 1-hydroxybenzotriazole (HOBt) as the activating reagents. Reverse Phase HPLC was performed on an Agilent 1200 series system equipped with an automated injector, UV-detector, fraction-collector and Agilent Zorbax Eclipse

XD8-C18 column (5 μm, 9.4 × 250 mm). The following gradient program was used for all purifications: water + 0.1% TFA over 5 min, then linear gradient 0-100% CH_3CN in water + 0.1% TFA 1.5 mL/min over 40 min. The NMR spectra were recorded on Varian Mercury (300, 500 MHz) spectrometers at 25˚C. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS, spectra are referenced by solvent signals. Coupling constants (*J*) are measured in Hertz (Hz) and are unadjusted. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, m – multiplet, br – broad. Various 2D NMR techniques (COSY, HSQC) were used to establish the structures and to assign the signals. Mass spectra were obtained using MALDI-ToF instruments (Applied Biosystems 4700 Proteomics Analyzer, Bruker Microflex LT Mass Spectrometer) with 2,5-dihydroxybenzoic acid or α-cyano-4-hydroxycinnamic acid as a matrix. Positive reflector mode was used unless stated otherwise. Monoisotopic masses are provided unless stated otherwise. Fragmentation was often observed in spectra of surface modified dendrimers, proposed fragments are displayed on the spectra. Sugar analysis was performed on DIONEX ICS-3000 HPAEC chromatograph using deionized water and 200 mM NaOH as an eluent. Sample preparation: 1-2 mg of sample and D-(+)-galactose were treated with 2M TFA in water (250 μl) for 4 h at 100˚C. Sample and standard were spin dried, redissolved in water (500 μl) and filtered. Sample concentration was then determined based on the calibration curves of galactose standards (1, 3, 10, 30, 50 μM). Average (of two measurements) number of galactose residues per molecule of dendrimer is provided.

Pent-4-ynoic anhydride (4): Compound **4** was prepared from pent-4-ynoic acid (3.8 g, 38.7mmol) as was described for compound **5**: **4** (3.1 g, 90%) colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00 (t, *J* = 2.6 Hz, 2H, 2×C≡CH), 2.52 (td, *J* = 7.3, 2.6 Hz, 4H,

2×CH_2CH_2C≡CH), 2.70 (t, J = 7.1 Hz, 4H, 2×C$H_2$$CH_2$C≡CH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.72 (2×CH_2C≡C), 34.27 (2×CH_2CH_2C≡C), 69.61 (2×C≡CH), 81.36 (2×CH_2C≡C), 167.10 (2×C=O).

5-(Trimethylsilyl)pent-4-ynoic acid: 5-(Trimethylsilyl)pent-4-yn-1-ol (5.0 g, 32 mmol) and pyridinium dichromate (30.0 g, 80 mmol) were dissolved in DMF (50 mL). The resulting black solution was stirred for 24 h. The reaction mixture was diluted with water (200 mL) and extracted with Et_2O (3×100 mL). The combined organic layers were washed with 1 M HCl (2×100 mL), brine (100 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated under the reduced pressure. The residue was purified by silica gel column chromatography (20 then 30% ethyl acetate in hexanes) to give 5-(Trimethylsilyl)pent-4-ynoic acid (3.5 g, 65%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.14 (s, 9H, $Si(CH_3)_3$), 2.51-2.64 (m, 4H, CH_2CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -0.01 ($Si(CH_3)_3$), 15.47 (CH_2C≡C), 33.33 (CH_2CH_2C≡C), 85.68 (CH_2C≡C), 104.51 (CH_2C≡C), 177.76 (C=O).

5-(Trimethylsilyl)pent-4-ynoic anhydride (5): 5-(Trimethylsilyl)pent-4-ynoic acid (3.0 g, 17.6 mmol) and *N,N*'-dicyclohexylcarbodiimide (DCC) (1.8 g, 8.8 mmol) were dissolved in DCM (50 mL). The resulting solution was stirred vigorously for 3 h. The reaction mixture was filtered and the filtrate was concentrated to approximately 10 mL. The filtrate was cooled (-20˚C) and kept for 1 h at this temperature. The resulting cloudy solution was filtered, the solvent evaporated under reduced pressure to give **5** (2.8 g, 98%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.13 (s, 18H, 2×$Si(CH_3)_3$), 2.54-2.73 (m, 8H, 2×CH_2CH_2); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -0.04 (2×$Si(CH_3)_3$), 15.22 (2×CH_2C≡C), 34.60 (2×CH_2CH_2C≡C), 86.13 (2×CH_2C≡C), 103.72 (2×CH_2C≡C), 167.27 (2×C=O).

Fluorescein azide (7): 5(6)-Carboxyfluorescein (451 mg, 1.2 mmol) and 2-[2-(2-azidoethoxy)-ethoxy]-ethylamine[25] **(3)** (174 mg, 1.0 mmol) were suspended in DCM (10 mL). (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) (624 mg, 1.2 mmol), HOBt (202 mg, 1.5 mmol) and *N,N*-diisopropylethylamine (DIPEA) (340 µL, 2.0 mmol, 2 equiv) were added to the mixture. After stirring for 48 h, glacial acetic acid (3 mL) was added and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (MeOH:DCM:AcOH 6:93:1 v/v/v, product containing fractions were further purified by using 50% acetone in hexanes) yielding an orange solid **7** (240 mg, 45%) as a mixture of 5- and 6-isomers: ^{1}H NMR (300 MHz, CD_6CO) δ 2.93 (br s, 4H, 4×OH of both isomers), 3.28 (t, J = 4.9 Hz, 2H, CH_2N$_3$ of 6- isomer), 3.37 (t, J = 4.7 Hz, 2H, CH_2N$_3$ of 5-isomer), 3.48-3.71 (m, 20H, CH_2NH of both isomers, 4×CH_2O of both isomers), 6.61-6.70 (m, 8H, 4×CH-aryl of both isomers), 6.76 (d, J = 2.2 Hz, 4H, 2×CH-aryl of both isomers), 7.37 (d, J = 8.1 Hz, 1H, CH-aryl of 5-isomer), 7.73 (s, 1H, CH-aryl of 6-isomer), 8.00-8.06 (m, 3H, NH of both isomers, CH-aryl of 6-isomer), 8.22 (dd, J = 8.0, 1.3 Hz, 1H, CH-aryl of 6-isomer), 8.30 (dd, J = 7.9, 1.4 Hz, 1H, CH-aryl of 6-isomer), 8.43 (s, 1H, CH-aryl of 5-isomer); ^{13}C NMR (only peaks of 6-isomer listed) (75.5 MHz, CD_6CO) δ 41.58 (CH_2NH), 52.26 (CH_2N_3), 71.01 (CH_2O), 71.62 (CH_2O), 71.89 (CH_2O), 71.98 (CH_2O), 104.34 (2×CH-aryl), 112.15 (2×C-aryl), 114.40 (2×CH-aryl), 124.30 (CH-aryl), 126.55 (CH-aryl), 131.12 (2×C-aryl), 131.29 (2×CH-aryl), 143.12 (C-aryl), 154.29 (2×C-aryl), 155.18 (C-aryl), 161.41 (2×C-aryl), 166.93 (C=O), 169.83 (C=O); MS (MALDI-ToF) Calc. for $C_{27}H_{25}N_4O_8$ $[M+H]^+$ 533.2, Found $[M+H]^+$ 533.2.

Azido-RGD peptide (8): Solid phase peptide synthesis was performed on Rink amide AM resin (0.2 mmol) using Fmoc-Asp(O*t*-Bu)-OH (1.0 mmol), Fmoc-Gly-OH (1.0 mmol), Fmoc-Arg(Pbf)-OH (1.0 mmol). Azidoacetic acid[34] (60 mg, 0.6 mmol) was coupled manually

using PyBop (310 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol), DIPEA (340 µL, 2 mmol). The resin was then thoroughly washed with DMF (10 mL), DCM (10 mL), and MeOH (10 mL) and dried *in vacuo*. The resin was then swelled in DCM (10 mL) for 1 h and treated with TFA:H_2O:TIS (95:2.5:2.5, v/v/v, 20 mL) for 2 h. The resin was filtered and washed with neat trifluoroacetic acid (TFA) (4 mL). The combined filtrates were concentrated *in vacuo* to approximately one third of the original volume. The crude peptide was precipitated by addition of Et_2O (0°C, 40 mL), recovered by centrifugation (5 °C, 3000 rpm, 20 min) and decanting of the solvent. The residue was purified by HPLC (t = 17.8 min). Lyophilization of the appropriate fractions gave peptide **8** (52 mg, 54%) as a white foam: MS (MALDI-ToF) Calc. for $C_{16}H_{28}N_{11}O_7$ $[M+H]^+$ 486.2, Found $[M+H]^+$ 486.2.

General procedure for the synthesis of dendrons 10, **11**, **12**, **13**, **14**, **18 and 21 by sequential isopropylidene acetal removal and ester formation:** Dowex® 50WX8-200 H^+ ion exchange resin (2-4 g) was added to the solution of isopropylidene-protected dendron (G1 to G4) in MeOH (10 mL) and the resulting suspension was stirred for 2-24 h at 50˚C. The reaction mixture was filtered and the resin was washed with MeOH (3×10mL). The combined filtrates were concentrated under reduced pressure to give hydroxyl-terminated dendron, which was used in the next step without further purification. Hydroxyl terminated dendron or amine **3**, DMAP and pyridine were dissolved in DCM (5-10 mL). The mixture was cooled to 0˚C and a solution of a suitable anhydride **1**, **4** or **5** in DCM (10-15 mL) was added to the mixture in small portions over 10 min. The reaction mixture was then allowed to warm to room temperature and stirred for 12-18 h. The solution was diluted with DCM (50-100 mL) washed with water (50-100 mL), sat. aq. $NaHCO_3$ (50-100 mL), sat aq. $CuSO_4$ (for anhydrides **1** and **5**, 50-100 mL) or 0.1 M HCl (for anhydride **4**, 2×50 mL) and brine (50-100 mL). The organic layer was dried ($MgSO_4$), filtered

and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel.

General procedure for the installation of a cyclooctynol moiety for the preparation of 16 and 20: Azido-containing dendron (0.06 mmol) was dissolved in a mixture of THF and H_2O (9:1, v/v, 5 mL). A 1 M solution of trimethylphosphine in THF (0.61 mL, 0.61 mmol) was added to the solution and the resulting mixture was stirred for 3 h. The solution was concentrated *in vacuo* and coevaporated with toluene (3×10 mL). The resulting yellow residue and 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester[20c] (**2**) (35 mg, 0.09 mmol) were dissolved in DMF (5 mL). DIPEA (0.04 mL, 0.24 mmol) was added to the solution and the resulting mixture was stirred for 48 h. Evaporation of the solvent under reduced pressure gave a residue, which was purified by silica gel column chromatography.

General procedure for Cu-free ligation of dendrons to give 17, 22, 23 and 28: Cyclooctynol-modified dendron and azido-containing dendron (or azide **7**) were dissolved in THF (10 μmol/mL) and the resulting mixture was stirred for 2-24 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography.

General procedure for CuAAC reactions using $CuSO_4$ and Na ascorbate for the preparation of 24, 25, 26 and 27: Alkynylated dendrimer, azide **6** or **9** and tris[(1-benzyl-1H-1,2,3 -triazol-4-yl)methyl]amine (TBTA) were dissolved in THF (5 μmol/mL of dendrimer). 0.1 M Solution of (+)-sodium L-ascorbate and 0.1 M solution of $CuSO_4$ in water were added to the mixture. The reaction mixture was stirred for 18 h. Solvent was evaporated and the residue was purified *via* HPLC. Fractions of interest were combined and lyophilized.

General procedure for CuAAC reaction using CuI for the preparation of 29 and 30: Dendrimer **28**, azide **6** or **9**, CuI and DIPEA were dissolved in THF (0.01 mmol/mL). The

reaction mixture was stirred for 4-20 h and the solvent was evaporated *in vacuo.* The residue was purified by SEC on Sephadex® LH-20 gel (MeOH:DCM, 1:1, v/v).

General procedure for CuF_2 mediated "click" reaction for the preparation of 31 and 32: Dendrimer **29**, azide **9** or azido-peptide **8** and CuF_2 were dissolved in MeOH (4 μmol/mL). The reaction mixture was stirred at 40˚C until completion of the reaction (monitored by MALDI-TOF MS). The solvent was evaporated and the residue was purified by HPLC to give after lyophilization of appropriate fractions the product.

G1 dendron (10): Prepared from 2-[2-(2-Azidoethoxy)-ethoxy]-ethylamine **(3)** (700 mg, 4.0 mmol) using: 4-dimethylaminopyridine (DMAP) (100 mg, 0.8 mmol), pyridine (1.6 mL, 20 mmol) and isopropylidene-2,2-bis(hydroxymethyl)propionic anhydride[22b, 35] **(1)** (2.64 g, 8.0 mmol) according to the general procedure for the synthesis of dendrons. The reaction mixture was stirred for 12 h. Flash chromatography on silica gel (30% acetone in hexanes) gave **10** (1.3 g, 99%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (s, 3H, CH_3), 1.40-1.44 (m, 6H, 2×CH_3), 3.35 (t, J =5.0 Hz, 2H, CH_2N_3), 3.46-3.74 (m, 12H, 5×OCH_2 , NHCH_2), 3.90 (d, J = 12.2 Hz, 2H, OCH_2), 7.37 (br s, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.73 (CH_3), 18.61 (CH_3), 28.42 (CH_3), 39.20 ($NHCH_2$), 40.14 (CH_2C), 50.58 (CH_2N_3), 67.05 (2×OCH_2C), 69.94 (CH_2O), 69.95 (CH_2O), 70.29 (CH_2O), 70.51 (CH_2O), 98.29 (C), 174.76 (C=O); MS (MALDI-ToF) Calc. for $C_{14}H_{27}N_4O_5$ $[M+H]^+$ 331.2, Found $[M+H]^+$ 331.2.

G2 dendron (11): G1 dendron **10** (1.25 g, 3.8 mmol) was unprotected using Dowex® 50WX8-200 H^+ resin (2 g, 2 h) to give hydroxyl terminated G1 dendron (1.1 g, 100%; MS (MALDI-ToF) Calc. for $C_{11}H_{23}N_4O_5$ $[M+H]^+$ 291.2, Found $[M+H]^+$ 291.2) as a colorless oil. G2 dendron **11** was prepared from hydroxyl terminated G1 dendron (1.05 g, 3.6 mmol) using: DMAP (170 mg, 1.4 mmol), pyridine (2.8 mL, 36.0 mmol) and anhydride **1** (5.0 g, 15.2 mmol)

according to the general procedure for the synthesis of dendrons. The reaction mixture was stirred for 18 h. Column chromatography on silica gel (30% acetone in hexanes) gave **11** (2.1 g, 92%) as a transparent oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 6H, 2×CH_3), 1.26 (s, 3H, CH_3), 1.35-1.41 (m, 12H, 4×CH_3), 3.35-3.67 (m, 16H, CH_2N_3, 6×OCH_2, NHCH_2), 4.15 (d, J = 11.8 Hz, 4H, 2×OCH_2), 4.25-4.35 (m, 4H, 2×OCH_2), 6.54 (br s, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.58 (CH_3), 18.36 (2×CH_3), 21.66 (2×CH_3), 25.51 (2×CH_3), 39.38 ($NHCH_2$), 42.09 (2×CH_2C), 46.59 (CH_2C), 50.58 (CH_2N_3), 65.99 (2×OCH$_2$C), 66.01 (2×OCH$_2$C), 66.19 (2×OCH$_2$C), 69.54 (CH_2O), 69.99 (CH_2O), 70.17 (CH_2O), 70.48 (CH_2O), 98.14 (2×C), 172.23(C=O), 173.54 (2×C=O); MS (MALDI-ToF) Calc. for $C_{27}H_{46}N_4O_{11}Na$ $[M+Na]^+$ 625.4, Found $[M+Na]^+$ 625.4.

G3 dendron (12): G2 dendron **11** (2.06 g, 3.40 mmol) was unprotected using Dowex® 50WX8-200 H^+ resin (3 g, 3 h) to give hydroxyl terminated G2 dendron (1.62 g, 91%; MS (MALDI-ToF) Calc. for $C_{21}H_{38}N_4O_{11}Na$ $[M+Na]^+$ 545.2, Found $[M+Na]^+$ 545.3) as a colorless oil. G3 dendron **12** was prepared from hydroxyl terminated G2 dendron (960 mg, 1.84 mmol) using: DMAP (134 mg, 1.10 mmol), pyridine (2.96 mL, 36.80 mmol) and anhydride **1** (4.86 g, 14.72 mmol) according to the general procedure for the synthesis of dendrons. The reaction mixture was stirred for 18 h. Column chromatography on silica gel (20% acetone in hexanes) gave **12** (2.00 g, 95%) as a pale yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.13 (s, 12H, 4×CH_3), 1.26-1.27 (m, 9H, 3×CH_3), 1.34 - 1.40 (m, 24H, 8×CH_3), 3.38 (t, J = 4.9 Hz, 2H, CH_2N_3), 3.46 (q, J = 5.1 Hz, 2H, NHCH_2), 3.56-3.68 (m, 16H, 8×OCH_2), 4.13 (d, J = 11.8 Hz, 8H, 4×OCH_2), 4.19 - 4.34 (m, 12H, 6×OCH_2), 6.39 (t, J = 5.3 Hz, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.55 (CH_3), 17.61 (2×CH_3), 18.48 (4×CH_3), 21.92 (2×CH_3), 21.94 (2×CH_3), 25.26 (4×CH_3), 39.45 ($NHCH_2$), 42.04 (4×CH_2C), 46.35 (CH_2C), 46.88 (2×CH_2C), 50.58 (CH_2N_3), 64.95 (4×OCH$_2$C), 65.91 (4×OCH$_2$C), 65.94 (4×OCH$_2$C), 67.12 (2×OCH$_2$C), 69.53 (CH_2O), 70.01

(CH_2O), 70.13 (CH_2O), 70.49 (CH_2O), 98.07 (4×C), 171.47 (C=O), 171.80 (2×C=O), 173.52 (4×C=O); MS (MALDI-ToF) Calc. for $C_{53}H_{86}N_4O_{23}Na$ $[M+Na]^+$ 1169.6, Found $[M+Na]^+$ 1169.8.

G4 dendron (13): G3 dendron **12** (1.20 g, 1.05 mmol) was unprotected using Dowex® 50WX8-200 H^+ ion-exchange resin (3 g, 12 h) to give hydroxyl terminated G3 dendron (1.04 g, 100%; MS (MALDI-ToF) Calc. for $C_{41}H_{70}N_4O_{23}Na$ $[M+Na]^+$ 1009.4, Found $[M+Na]^+$ 1009.7) as an amorphous solid. G4 dendron **13** was prepared from hydroxyl terminated G3 dendron (430 mg, 0.44 mmol) using: DMAP (54 mg, 0.44 mmol), pyridine (1.42 mL, 17.60 mmol) and anhydride **1** (2.32 g, 7.04 mmol) according to the general procedure for the synthesis of dendrons. The reaction mixture was stirred for 18 h. Column chromatography (30% acetone in hexanes) gave **13** (930 mg, 94%) as a yellowish oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12 (s, 24H, 8×CH_3), 1.24-1.27 (m, 21H, 7×CH_3), 1.32 - 1.39 (m, 48H, 16×CH_3), 3.37 (t, J = 5.2 Hz, 2H, CH_2N_3), 3.43 (q, J = 5.2 Hz, 2H, NHCH_2), 3.54-3.67 (m, 24H, 12×OCH_2), 4.12 (d, J = 11.9 Hz, 16H, 8×OCH_2), 4.17 - 4.32 (m, 28H, 14×OCH_2), 6.49 (t, J = 5.5 Hz, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.37 (CH_3), 17.41 (2×CH_3), 17.62 (4×CH_3), 18.44 (8×CH_3), 22.00 (8×CH_3), 25.12 (8×CH_3), 39.43 ($NHCH_2$), 41.97 (8×CH_2C), 46.31 (CH_2C), 46.66 (2×CH_2C), 46.76 (4×CH_2C), 50.52 (CH_2N_3), 64.74 (8×OCH_2C), 65.54 (4×OCH_2C), 65.84 (8×OCH_2C), 65.89 (8×OCH_2C), 67.34 (2×OCH_2C), 69.51 (CH_2O), 69.94 (CH_2O), 70.03 (CH_2O), 70.42 (CH_2O), 98.02 (8×C), 171.27 (C=O), 171.35 (2×C=O), 171.78 (4×C=O), 173.42 (8×C=O); MS (MALDI-ToF) Calc. for $C_{105}H_{166}N_4O_{47}Na$ $[M+Na]^+$ 2258.1, Found $[M+Na]^+$ 2258.7.

G3 dendron (14): G3 dendron **14** was prepared from hydroxyl terminated G3 dendron (500 mg, 0.50 mmol) using: DMAP (61 mg, 0.50 mmol), pyridine (1.45 mL, 18 mmol) and pent-4-ynoic anhydride (**4**) (1.07 g, 6 mmol) according to the general procedure for the synthesis of

dendrons. The reaction mixture was stirred for 15 h. Column chromatography (30% acetone in hexanes) gave **14** as a colorless oil (724 mg, 89%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23-1.27 (m, 21H, 7×CH_3), 1.98 (t, J = 2.4 Hz, 8H, 8×C≡CH), 2.46 (td, J = 6.4, 1.8 Hz, 16H, 8×CH_2C≡CH), 2.54 (t, J = 7.0 Hz, 16H, 8×C$H_2$$CH_2$C≡CH), 3.37 (t, J = 4.9 Hz, 2H, CH_2N_3), 3.44 (q, J = 4.9 Hz, 2H, NHCH_2), 3.55 (t, J = 5.0 Hz, 2H, C$H_2$$CH_2N_3$), 3.62-3.67 (m, 6H, 3×$OCH_2$), 4.19 - 4.25 (m, 28H, 14×OCH_2), 6.51 (t, J = 5.0 Hz, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.20 (8×*C*H_2C≡CH), 17.44 (3×CH_3), 17.72 (4×CH_3), 33.05 (8×*C*$H_2$$CH_2$C≡CH), 39.43 (NHC$H_2$), 46.24 ($CH_2$*C*), 46.29 (4×$CH_2$*C*), 46.66 (2×$CH_2$*C*), 50.50 ($CH_2N_3$), 65.18 (8×O*C*$H_2$C), 65.31 (4×O*C*$H_2$C), 67.14 (2×O*C*$H_2$C), 69.27 (8×C≡*C*H), 69.51 (CH_2O), 69.91 (CH_2O), 70.00 (CH_2O), 70.38 (CH_2O), 82.24 (8×*C*≡CH), 171.07 (8×C=O), 171.30 (C=O), 171.40 (2×C=O), 171.85 (4×C=O); MS (MALDI-ToF) Calc. for $C_{81}H_{102}N_4O_{31}Na$ $[M+Na]^+$ 1649.6, Found $[M+Na]^+$ 1649.9.

G3 dendron (16): Prepared from G3 azido-containing dendron **14** (100 mg, 0.06 mmol) according to the general procedure for the installation of a cyclooctynol moiety. Silica gel column chromatography (gradient 20 to 50% acetone in hexanes) gave **16** (101 mg, 89%) as a viscous colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27-1.27 (m, 21H, 7×CH_3), 1.98-1.99 (m, 8H, 8×C≡CH), 2.46-2.56 (m, 32H, 8×CH_2C≡CH, 8×C$H_2$$CH_2$C≡CH), 2.89 (dd, J = 15.1, 3.0 Hz, 1H, CH*H*CH), 3.16 (d, J = 15.1 Hz, 1H, C*H*HCH), 3.39-3.63 (m, 12H, 2×NHCH_2, 4×OCH_2), 4.20 - 4.26 (m, 28H, 14×OCH_2), 5.48-5.50 (m, 2H, CH_2C*H*, NHCOO) 6.49 (m, 1H, NHCO), 7.26-7.34 (m, 7H, 7 × CH-aryl), 7.49 (d, J = 7.4 Hz, 1H, CH-aryl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.25 (8×*C*H_2C≡CH), 17.49 (3×CH_3), 17.78 (4×CH_3), 33.10 (8×*C*$H_2$$CH_2$C≡CH), 39.43 (NHC$H_2$), 40.86 (NHC$H_2$), 46.13 (*C*$H_2$CH), 46.27 ($CH_2$*C*), 46.34 (4×$CH_2$*C*), 46.69 (2×$CH_2$*C*), 65.22 (8×O*C*$H_2$C), 65.34 (4×O*C*$H_2$C), 67.11 (2×O*C*$H_2$C), 69.32 (8×C≡*C*H), 69.46 (CH_2O),

70.01 (CH_2O), 70.08 (CH_2O), 70.17 (CH_2O), 76.83 (CH_2CH), 82.27 (8×C≡CH), 109.88 (C≡C), 112.91 (C≡C), 121.28 (C-aryl), 123.62 (CH-aryl), 123.77 (C-aryl), 125.95 (CH-aryl), 126.25 (CH-aryl), 127.05 (CH-aryl), 127.07 (CH-aryl), 127. 86 (CH-aryl), 127.99 (CH-aryl), 129.85 (CH-aryl), 150.93 (C=O), 152.03 (C-aryl), 155.47 (C-aryl), 171.11 (8×C=O), 171.43 (3×C=O), 171.89 (4×C=O); MS (MALDI-ToF) Calc. for $C_{98}H_{114}N_2O_{33}Na$ $[M+Na]^+$ 1869.7, Found $[M+Na]^+$ 1870.2.

Fluorescein-labeled dendrimer 17 (isomers): Prepared from G3 dendron **16** (10.0 mg, 5.4 μmol) and fluorescein azide **7** (3.2 mg, 6.0 μmol) according to the general procedure for Cu-free ligation of dendrons. The reaction mixture was stirred for 2 h. Silica gel column chromatography (5% MeOH in DCM) gave dendrimer **17** (12.8 mg, 95%) as an orange oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20-1.38 (m, 21H, 7×CH_3), 1.63-1.99 (m, 10H, 8×C≡CH, 2×OH), 2.47-2.64 (m, 32H, 8×CH_2C≡CH, 8×CH_2CH_2C≡CH), 2.97-4.51 (m, 54H, CH_2CH, 6×OCH_2CH_2, 14×OCH_2), 5.50-5.68 (m, 1H, NH), 5.87-6.17 (m, 1H, CH_2CH), 6.32-8.44 (m, 19H, 17×CH-aryl, 2×NH); MS (most abundant mass) (MALDI-ToF) Calc. for $C_{125}H_{138}N_6O_{41}Na$ $[M+Na]^+$ 2402.9, Found $[M+Na]^+$ 2402.4.

G4 dendron (18): G4 dendron **13** (930 mg, 0.42 mmol) was unprotected using Dowex® 50WX8-200 H^+ resin (4 g, 24 h) to give hydroxyl terminated G4 dendron (790 mg, 98%; MS (MALDI-ToF) Calc. for $C_{81}H_{134}N_4O_{47}Na$ $[M+Na]^+$ 1937.8, Found $[M+Na]^+$ 1938.1) as a white foam. G4 dendron **18** was prepared from hydroxyl terminated G4 dendron (785 mg, 0.41 mmol) using: DMAP (150 mg, 1.23 mmol), pyridine (2.64 mL, 32.8 mmol) and anhydride **4** (1.75 g, 9.84 mmol) according to the general procedure for the synthesis of dendrons. The reaction mixture was stirred for 18 h. Silica gel column chromatography (40% acetone in hexanes) gave **18** (965 mg, 74%) as a viscous colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25-1.32 (m, 45H,

15×CH_3), 2.00 (t, J = 2.5 Hz, 16H, 16×C≡CH), 2.47 (td, J = 6.8, 2.1 Hz, 32H, 16×CH_2C≡CH), 2.56 (t, J = 6.9 Hz, 32H, 16×C$H_2$$CH_2$C≡CH), 3.39 (t, J = 5.0 Hz, 2H, CH_2N_3), 3.44 (q, J = 5.1 Hz, 2H, NHCH_2), 3.57 (t, J = 5.2 Hz, 2H, C$H_2$$CH_2N_3$), 3.62-3.68 (m, 6H, 3×$OCH_2$), 4.18 - 4.30 (m, 60H, 30×OCH_2), 6.53 (t, J = 5.4 Hz, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.27 (16×*C*H_2C≡CH), 17.24 (CH_3), 17.40 (2×CH_3), 17.52 (4×CH_3), 17.80 (8×CH_3), 33.11 (16×*C*$H_2$$CH_2$C≡CH), 39.50 ($NHCH_2$), 46.34 (8×$CH_2$*C*), 46.40 ($CH_2$*C*), 46.64 (2×$CH_2$*C*), 46.67 (4×$CH_2$*C*), 50.55 ($CH_2N_3$), 65.23 (24×O*C*$H_2$C), 65.80 (4×O*C*$H_2$C), 67.69 (2×O*C*$H_2$C), 69.35 (16×C≡*C*H), 69.54 (CH_2O), 69.97 (CH_2O), 70.05 (CH_2O), 70.42 (CH_2O), 82.32 (16×*C*≡CH), 171.11 (16×C=O), 171.25 (C=O), 171.30 (2×C=O), 171.46 (4×C=O), 171.88 (8×C=O); MS (MALDI-ToF) Calc. for $C_{161}H_{198}N_4O_{63}Na$ $[M+Na]^+$ 3218.2g/mol, Found $[M+Na]^+$ 3219.5.

G4 dendron (20): Prepared from G4 azido-containing dendron **18** (200 mg, 0.06 mmol) according to the general procedure for the installation of a cyclooctynol moiety. Silica gel column chromatography (40 then 50% acetone in hexanes) gave **20** (198 mg, 93%) as a viscous colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22-1.29 (m, 45H, 15×CH_3), 1.96-2.00 (m, 16H, 16×C≡CH), 2.43 - 2.54 (m, 64H, 16×CH_2CH_2C≡CH), 2.86 (dd, J = 15.0, 3.0 Hz, 1H, CH*H*CH), 3.13 (d, J = 14.8 Hz, 1H, C*H*HCH), 3.36-3.42 (m, 4H, 2×NHCH_2), 3.54-3.61 (m, 8H, 4×OCH_2), 4.18 - 4.24 (m, 60H, 30×OCH_2), 5.45-5.53 (m, 2H, CH_2C*H*, NHCOO), 6.55-6.60 (m, 1H, NHCO), 7.24-7.32 (m, 7H, 7×CH-aryl), 7.46 (d, J = 7.5, 1H, CH-aryl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.17 (16×*C*H_2C≡CH), 17.21 (CH_3), 17.32 (2×CH_3), 17.42 (4×CH_3), 17.70 (8×CH_3), 33.01 (16×*C*$H_2$$CH_2$C≡CH), 39.38 ($NHCH_2$), 40.76 ($NHCH_2$), 46.25 (*C*$H_2$CH, 8×$CH_2$*C*), 46.52 (3×$CH_2$*C*), 46.57 (4×$CH_2$*C*), 65.13 (24×O*C*$H_2$C), 65.67 (4×O*C*$H_2$C), 67.44 (2×O*C*$H_2$C), 68.03 ($CH_2O$), 69.30 (16×C≡*C*H), 69.95 (CH_2O), 70.06 (CH_2O), 70.55 (CH_2O), 76.71 (CH_2*C*H), 82.24 (16×*C*≡CH), 109.79 (*C*≡C), 112.83 (C≡*C*), 121.18 (C-aryl), 123.56 (CH-aryl), 123.67 (C-aryl),

125.87 (CH-aryl), 126.18 (CH-aryl), 126.99 (2×CH-aryl), 127. 78 (CH-aryl), 127.92 (CH-aryl), 129.79 (CH-aryl), 150.85 (C=O), 151.95 (C-aryl), 155.38 (C-aryl), 171.01 (16×C=O), 171.21 (3×C=O), 171.38 (4×C=O), 171.78 (8×C=O); MS (MALDI-ToF) Calc. for $C_{178}H_{210}N_2O_{65}Na$ $[M+Na]^+$ 3438.3, Found $[M+Na]^+$ 3439.5.

G2 dendron (21): G2 dendron **21** was prepared from hydroxyl terminated G2 dendron (637 mg, 1.22 mmol) using: DMAP (74 mg, 0.61 mmol), pyridine (1.97 mL, 24.40 mmol) and 5-(trimethylsilyl)pent-4-ynoic anhydride **(5)** (2.36 g, 7.32 mmol) according to the general procedure for the synthesis of dendrons. The reaction mixture was stirred for 18 h. Silica gel column chromatography (20% acetone in hexanes) gave **21** (1.26 g, 91%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 36H, 4×$Si(CH_3)_3$), 1.17 (s, 9H, 3×CH_3), 2.39-2.51 (m, 16H, 4×$CH_2CH_2C{\equiv}C$), 3.31 (t, J = 5.0 Hz, 2H, CH_2N_3), 3.38 (q, J = 4.9 Hz, 2H, NHCH_2), 3.50 (t, J = 4.9 Hz, 2H, C$H_2CH_2N_3$), 3.57-3.62 (m, 6H, 3×OCH_2), 4.16 (s, 12H, 6×OCH_2), 6.34 (t, J = 5.0 Hz, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ -0.16 (4×$Si(CH_3)_3$), 15.42 (4×$CH_2C{\equiv}C$), 17.44 (CH_3), 17.50 (2×CH_3), 33.14 (4×$CH_2CH_2C{\equiv}C$), 39.25 ($NHCH_2$), 46.12 (CH_2C), 46.34 (2×CH_2C), 50.39 (CH_2N_3), 65.02 (4×OCH_2C), 66.64 (2×OCH_2C), 69.33 (CH_2O), 69.83 (CH_2O), 69.93 (CH_2O), 70.29 (CH_2O), 85.23 (4×$CH_2C{\equiv}C$), 104.55 (4×$CH_2C{\equiv}C$), 170.93 (4×C=O), 171.29 (C=O), 171.65 (2×C=O); MS (MALDI-ToF) Calc. for $C_{53}H_{86}N_4O_{15}NaSi_4$ $[M+Na]^+$ 1153.5, Found $[M+Na]^+$ 1153.6.

Dendrimer 22 (isomers): Prepared from G3 dendron **16** (20.0 mg, 11 μmol) and G3 dendron **14** (19.7 mg, 12.0 μmol) according to the general procedure for Cu-free ligation of dendrons. The reaction mixture was stirred for 11 h. Silica gel column chromatography (50% acetone in hexanes) gave **32** (36.0 mg, 94%) as a viscous oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23-1.29 (m, 42H, 14×CH_3), 1.88-1.98 (m, 16H, 16×C≡CH), 2.46-2.55 (m, 64H,

16×CH_2C≡CH, 16×CH_2CH$_2$C≡CH), 2.98-3.71 (m, 22H, CH_2CH, 2×OCH_2CH_2, 3×NHCH_2CH_2), 3.97-4.59 (m, 60H, 28×OCH$_2$, OCH_2CH_2-triazole), 5.15-5.40 (m, 1H, NHCOO), 5.94-6.18 (m, 1H, CH$_2$C*H*), 6.46-6.76 (m, 2H, 2×NHCO), 7.10-7.55 (m, 8H, 8×CH-aryl); MS (MALDI-ToF) Calc. for $C_{179}H_{216}N_6O_{64}Na$ $[M+Na]^+$ 3496.4, Found $[M+Na]^+$ 3497.8.

Dendrimer 23 (isomers): Prepared from G4 dendron **20** (34.2 mg, 10.0 μmol) and G4 dendron **18** (35.2 mg, 11.0 μmol) according to the general procedure for Cu-free ligation of dendrons. The reaction mixture was stirred for 24 h. Silica gel column chromatography (3% MeOH in DCM) gave dendrimer **23** (61 mg, 93%) as a viscous oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30-1.41 (m, 90H, 30×CH$_3$), 2.36-2.82 (m, 160H, 32×C≡CH, 32×CH_2C≡CH, 32×CH_2CH$_2$C≡CH), 2.98-3.75 (m, 22H, CH_2CH, 2×OCH_2CH_2, 3×NHCH_2CH_2), 3.98-4.73 (m, 124H, 60×OCH$_2$, OCH_2CH_2-triazole), 5.96-6.46 (m, 2H, NHCOO, CH$_2$C*H* ,), 7.10-7.55 (m, 10H, 8×CH-aryl, 2×NHCO); MS (most abundant mass) (MALDI-ToF) Calc. for $C_{339}H_{408}N_6O_{128}Na$ $[M+Na]^+$ 6637.6, Found $[M+Na]^+$ 6643.2.

Glycodendrimer 24 (isomers): Prepared from dendrimer **17** (10.0 mg, 4.2 μmol) using: 3-azidopropyl β-D-galactopyranoside[28] (**9**) (17.7 mg, 67.2 μmol), TBTA (3.6 mg, 6.7 μmol), $CuSO_4$ (67 μL of 0.1 M solution in water, 6.7 μmol) and (+)-sodium L-ascorbate (168 μL of 0.1 M solution in water, 16.8 μmol) according to the general procedure for CuAAC reactions using $CuSO_4$ and Na ascorbate. HPLC purification (t = 26.6 min) gave **24** (17.3 mg, 92%) as a yellow powder: ^{1}H NMR (500 MHz, D_2O) δ 1.03-1.25 (m, 21H, 7×CH$_3$), 2.04-2.21 (m, 16H, 8×CH$_2$CH_2CH$_2$-triazole), 2.60-2.99 (m, 32H, 8×CH_2CH_2-triazole), 3.09-4.45 (m, 142H, 8×CH_2CH$_2$CH_2, 40×CH of galactose, 8×CH$_2$ of galactose, CH_2CH, 6×CH_2CH_2O, 14×OCH$_2$), 5.65-5.93 (m, 1H, CH$_2$C*H*), 6.27-8.31 (m, 25H, 8×CH-aryl of cyclooctyne, 9×CH-aryl of

fluorescein, 8×CH of triazole); Sugar analysis: Calc. 8, Found 10.38 ± 0.56; MS (most abundant mass) (MALDI-ToF) Calc. for $C_{197}H_{274}N_{30}O_{89}Na$ $[M+Na]^+$ 4508.8, Found $[M+Na]^+$ 4510.0.

Glycodendrimer 25 (isomers): Prepared from dendrimer **22** (17.4 mg, 5.0 μmol) using: 3-azidopropyl β-D-galactopyranoside (**9**) (42.0 mg, 160.0 μmol), TBTA (8.6 mg, 16.0 μmol), $CuSO_4$ (160 μL of 0.1 M solution in water, 16.0 μmol) and (+)-sodium L-ascorbate (400 μL of 0.1 M solution in water, 40.0 μmol) according to the general procedure for CuAAC reactions using $CuSO_4$ and Na ascorbate. HPLC purification (t = 24.9 min) gave **25** (34.6 mg, 90%) as a white powder.^{1}H NMR (500 MHz, D_2O) δ 1.08-1.28 (m, 42H, 14×CH_3), 2.13-2.25 (m, 32H, 16×$CH_2CH_2CH_2$-triazole), 2.64-3.10 (m, 64H, 16×CH_2CH_2-triazole), 3.30-4.66 (m, 258H, 16×$CH_2CH_2CH_2$, 64×CH of galactose, 16×CH_2 of galactose, CH_2CH, 6×CH_2CH_2O, 28×OCH_2), 5.82-6.04 (m, 1H, CH_2CH), 7.11-7.59 (m, 8H, 8×CH-aryl of cyclooctyne), 7.88-8.04 (m, 16H, 16×CH of triazole); Sugar analysis: Calc. 16, Found 15.10 ± 0.83; MS (MW, linear mode), (MALDI-ToF) Calc. for $C_{323}H_{489}N_{54}O_{160}$ $[M+H]^+$ 7688.6, Found $[M+H]^+$ 7685.5.

Dendrimer 26 (isomers): Prepared from dendrimer **23** (10.0 mg, 1.5 μmol) using: 2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]-ethanol[27] (**6**) (21.2 mg, 96.6 μmol), TBTA (5.1 mg, 9.6 μmol), $CuSO_4$ (96 μL of 0.1 M solution in water, 9.6 μmol) and (+)-sodium L-ascorbate (240 μL of 0.1 M solution in water, 24.0 μmol) according to the general procedure for CuAAC reactions using $CuSO_4$ and Na ascorbate. HPLC purification (t = 32.5 min), followed by preparative SEC on Sephadex® LH-20 gel (MeOH:DCM, 1:1, v/v), gave **26** as a transparent glass (11 mg, 53%): ^{1}H NMR (500 MHz, D_2O) δ 1.09-1.35 (m, 90H, 30×CH_3), 2.68-2.79 (m, 64H, 32×CH_2CH_2-triazole), 2.90-3.01 (m, 64H, 32×CH_2CH_2-triazole), 3.45-3.76 (m, 406H, 98×OCH_2CH_2O, 3×NH CH_2CH_2, CH_2CH of cyclooctyne), 3.86-3.95 (m, 66H, 33×OCH_2CH_2-triazole), 4.05-4.39 (m, 120H, 60×CH_2O), 4.48-4.61 (m, 66H, 33×OCH_2CH_2 –triazole), 5.85-6.10 (m, 1H, CH_2CH),

7.14-7.61 (m, 8H, 8×CH-aryl of cyclooctyne), 7.81 (br s 32H, 32×CH of triazole); MS (MW, linear mode) (MALDI-ToF) Calc. for $C_{595}H_{953}N_{102}O_{256}$ $[M+H]^+$ 13631.5, Found $[M+H]^+$ 13643.7.

Glycodendrimer 27 (isomers): Prepared from dendrimer **23** (10.0 mg, 1.5 μmol) using: 3-azidopropyl β-D-galactopyranoside[28] (**9**) (25.4 mg, 96.6 μmol), TBTA (5.1 mg, 9.6 μmol), $CuSO_4$ (96 μL of 0.1 M solution in water, 9.6 μmol) and (+)-sodium L-ascorbate (240 μL of 0.1 M solution in water, 24.0 μmol) according to the general procedure for CuAAC reactions using $CuSO_4$ and Na ascorbate. HPLC purification (t = 24.0 min) gave **27** (19 mg, 84%) as a white powder: ^{1}H NMR (500 MHz, D_2O) δ 0.99-1.37 (m, 90H, 30×CH_3), 2.11-2.16 (m, 64H, 32×$CH_2CH_2CH_2CH_2$-triazole), 2.73 (br s, 64H, 32×CH_2CH_2–triazole), 2.97 (br. s, 64H, 32×CH_2CH_2–triazole), 3.48-3.76 (m, 216H, 32×$CHCH_2CH_2$-triazole, 32×CH_2 of galactose, 96×COH of galactose, 5×CH_2CH_2O, OCH_2CH_2-triazole, CH_2CH of cyclooctyne), 3.84-3.90 (m, 64H, 32×$CHHCH_2CH_2$-triazole, 32×COH of galactose), 4.00-4.33 (m, 152H, 32×CHO of galactose, 60×CH_2O), 4.47-4.62 (m, 66H, $CH_2CH_2CH_2$ –triazole, OCH_2CH_2-triazole) 5.79-6.05 (m, 1H, CH_2CH), 7.11-7.57 (m, 8H, 8×CH-aryl of cyclooctyne), 7.99(br. s 32H, 32×CH-triazole). Sugar analysis: 31.77 ± 0.18 (requires 32).

Dendrimer 28 (isomers): Prepared from G3 dendron **16** (36.9 mg, 20 μmol) and G2 dendron **21** (25.0 mg, 22 μmol) according to the general procedure for Cu-free ligation of dendrons. The reaction mixture was stirred for 5 h. Silica gel column chromatography (40% acetone in hexanes) gave dendrimer **28** (53 mg, 90%) as viscous oil: ^{1}H NMR (500 MHz, CD_6CO, 25˚C, TMS): δ= 7.68-7.15 (m, 10 H, CH, NH), 6.55-6.46 (m, 1 H, NH), 6.22-5.97 (m, 1 H, CH), 4.65-4.56 (m, 2 H, CH_2), 4.34-4.28 (m, 40 H, CH_2), 4.01-3.79 (m, 2 H, CH_2), 3.79-2.79 (m, 22 H, CH_2), 2.60-2.46 (m, 48 H, CH_2), 2.36 (s, 8 H, CH), 1.37-1.28 (m, 30 H, CH_3), 0.11

ppm (s, 36 H, CH_3); MS (MALDI-TOF): *m/z*: calcd for $C_{151}H_{200}N_6O_{48}NaSi_4$: 3000.2 $[M+Na]^+$; found: 3001.3.

Dendrimer 29 (isomers): Prepared from dendrimer **28** (30.0 mg, 10 μmol) using: 2-[2-[2-(2-azidoethoxy)ethoxy]ethoxy]-ethanol (**6**) (26.3 mg, 120 μmol), CuI (1.5 mg, 8 μmol) and DIPEA (7 μL, 40 μmol) according to the general procedure for CuAAC reaction using CuI. The reaction mixture was stirred for 4 h. SEC purification gave **29** as transparent oil (41 mg, 87%): ^{1}H NMR (500 MHz, CD_6CO) δ 0.11 (s, 36H, 4×$Si(CH_3)_3$), 1.22-1.38 (m, 30H, 10×CH_3), 2.50-2.57 (m, 16H, 4×CH_2CH_2C≡C), 2.74 (t, J = 7.4 Hz, 16H, 8×C$H_2$$CH_2$-triazole), 2.97 (t, J = 7.4 Hz, 16H, 8×CH_2CH_2-triazole), 3.24-4.02 (m, 136H, CH_2CH, 26×OCH_2CH_2O, 9×OC$H_2$$CH_2$-triazole, 3×NHC$H_2CH_2$O), 4.19-4.37 (m, 40H, 20×OCH_2), 4.52 (t, J = 5.0 Hz, 18H, 9×OCH_2CH_2-triazole), 5.96-6.24 (m, 1H, CH_2CH), 6.31-6.70 (m, 1H, NH), 7.19-7.68 (m, 10H, 8×CH-aryl, 2×NH), 7.79 (s, 8H, 8×CH of triazole); MS (most abundant mass) (MALDI-ToF) Calc. for $C_{215}H_{336}N_{30}O_{80}Si_4Na$ $[M+Na]^+$ 4756.2, Found $[M+Na]^+$ 4759.2.

Dendrimer 30 (isomers): Prepared from dendrimer **25** (15.0 mg, 5.0 μmol) using: 3-azidopropyl β-D-galactopyranoside (**9**) (21.0 mg, 80.0 μmol), CuI (0.8 mg, 4.5 μmol) and DIPEA (4 μL, 20.0 μmol) according to the general procedure for CuAAC reaction using CuI. The reaction mixture was stirred for 20 h. SEC purification gave **30** as transparent oil (19 mg, 75%): ^{1}H NMR (500 MHz, CD_3OD, 25˚C, TMS): δ= 7.83 (s, 8 H, CH), 7.66-7.19 (m, 8 H, CH), 6.15-5.92 (m, 1 H, CH), 4.60-4.50 (m, 18 H, CH_2), 4.25-4.10 (m, 48 H, CH_2, CH), 4.01-3.24 (m, 88 H, CH_2, CH), 3.01-2.91 (m, 16 H, CH_2), 2.81-2.67 (m, 16 H, CH_2), 2.57-2.44 (m, 16 H, CH_2), 2.21-2.08 (m, 16 H, CH_2), 1.31-1.12 (m, 30 H, CH_3), 0.10 (s, 36 H, CH_3); MS (MALDI-TOF, most abundant mass): *m/z*: calcd for $C_{223}H_{336}N_{30}O_{96}Si_4Na$: 5108.1 $[M+Na]^+$; found: 5108.3.

Dendrimer 31 (isomers): Prepared from dendrimer **29** (5.0 mg, 1.06 μmol) using 3-azidopropyl β-D-galactopyranoside **(9)** (2.2 mg, 8.4 μmol) and CuF_2 (0.8 mg, 8.4 μmol) according to the general procedure for CuF_2 mediated "click" reaction. The reaction mixture was stirred for 8 h. HPLC purification (t = 28.5 min) gave **31** (4.9 mg, 84%) as a white foam: ^{1}H NMR (500 MHz, D_2O) δ 1.04-1.35 (m, 30H, 10×CH_3), 2.09-2.23 (m, 8H, 4×$OCH_2CH_2CH_2$-triazole), 2.66-2.78 (m, 24H, 12×CH_2CH_2-triazole), 2.91-3.02 (m, 24H, 12×CH_2CH_2-triazole), 3.18-3.79 (m, 138H, CH_2CHO, 3×OCH_2CH_2NH, 26×OCH_2CH_2O, 12×CHOH of galactose, 4×CH_2 of galactose, 4×$OCHCH_2CH_2$-triazole), 3.84-3.99 (m, 30H, 4×$OCHCH_2CH_2$-triazole, 4×CHOH of galactose, 9×OCH_2CH_2-triazole), 4.02-4.39 (m, 44H, 4×CHO of galactose, 20×OCH_2), 4.47-4.66 (m, 26H, 13×CH_2-triazole), 5.80-6.13 (m, 1H, CH_2CH), 7.12-7.68 (m, 8H, 8×CH-aryl), 7.86-7.89 (m, 12H, 12×CH of triazole); MS (most abundant mass) (MALDI-ToF) Calc. for $C_{239}H_{372}N_{42}O_{104}Na$ $[M+Na]^+$ 5519.5, Found $[M+Na]^+$ 5524.3.

Dendrimer 32 (isomers): Prepared from dendrimer **29** (10.0 mg, 2.1 μmol) using azido-RGD peptide **8** (6.1 mg, 12.6 μmol) and CuF_2 (1.7 mg, 16.8 μmol) according to the general procedure for CuF_2 mediated "click" reaction. The reaction mixture was stirred for 19 h. HPLC purification (t = 26.9 min) gave **32** (8.4 mg, 63%) as a white foam. ^{1}H NMR (500 MHz, D_2O) δ 1.05-1.32 (m, 30H, 10×CH_3), 1.57-1.69 (m, 8H, 4×α-$CHCH_2CH_2CH_2$), 1.73-1.80 (m, 4H, 4×α-$CHCHCH_2CH_2$), 1.85-1.93 (m, 4H, 4×α-$CHCHCH_2CH_2$), 2.68-2.76 (m, 24H, 12×CH_2CH_2-triazole), 2.83-2.99 (m, 32H, 12×CH_2CH_2-triazole, 4×CH_2COOH), 3.18 (t, J = 6.9 Hz, 8H, 4×α-$CHCH_2CH_2CH_2$), 3.24-3.75 (m, 118H, CH_2CHO, 3×OCH_2CH_2NH, 26×OCH_2CH_2O), 3.87-4.35 (m, 78H, 9×OCH_2CH_2-triazole, 8×$NHCH_2$ of Gly, 4×α-$CHCH_2CH_2CH_2$, 20×OCH_2), 4.51-4.56 (m, 18H, 9×CH_2-triazole), 4.73 (dd, J = 7.4, 5.3 Hz, 4H, 4×α-CH of Asp), 5.27 (s, 8H, 4×triazole-CH_2 of peptide), 5.83-6.09 (m, 1H, CH_2CH), 7.13-7.61 (m, 8H, 8×CH-aryl), 7.81(s,

12H, 12×CH of triazole); MS (MW, linear mode) (MALDI-ToF) Calc. for $C_{267}H_{413}N_{74}O_{108}$ $[M+H]^+$ 6387.6, Found $[M+H]^+$ 6372.6.

Dendrimer 33 (isomers): Dendrimer **27** (13.0 mg, 2.6 μmol), azido-RGD peptide **8** (7.5 mg, 15.4 μmol) and CuF_2 (2.1 mg, 20.8 μmol) were dissolved in MeOH:H_2O mixture 1:1 v/v (0.5 mL). The reaction mixture was stirred for 40 h at 40˚C and the solvent was evaporated. The residue was purified by HPLC (t = 23.7 min) to give after lyophilization **33** (10.0 mg, 57%) as a white foam. ^{1}H NMR (500 MHz, D_2O, 25˚C, TMS): δ= 7.81 (s, 12 H, CH), 7.59-7.11 (m, 8 H, CH), 6.12-5.80 (m, 1 H, CH), 5.27 (s, 8 H, CH_2), 4.73 (dd, ^3J(H,H) = 7.2, 5.6 Hz, 4 H, CH), 4.52-4.43 (m, 18 H, CH_2), 4.37-3.84 (m, 84 H, CH_2, CH), 3.79-3.25 (m, 72 H, CH_2, CH), 3.17 (t, ^3J(H,H) = 6.8 Hz, 8 H, CH_2), 2.99-2.83 (m, 32 H, CH_2), 2.79-2.62 (m, 24 H, CH_2), 2.21-2.06 (m, 16 H, CH_2), 1.92-1.72 (m, 8 H, CH_2), 1.70-1.56 (m, 8 H, CH_2), 1.32-0.99 (m, 30 H, CH_3); MS (MALDI-TOF, MW, linear mode): *m/z*: calcd for $C_{275}H_{413}N_{74}O_{124}$ $[M+H]^+$ = 6739.6; found: 6720.7.

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CHAPTER 4

MULTI-FUNCTIONALIZATION OF POLYMERS BY STRAIN-PROMOTED CYCLOADDITIONS[†]

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Abstract

A unique synthetic route to oxime and azide bearing block copolymers was developed *via* reversible addition-fragmentation chain transfer copolymerization of 4-vinylbenzaldehyde and 1-(chloromethyl)-4-vinylbenzene with styrene, which made it possible to prepare in a controlled manner multi-functional polymers that could self-assemble into well-defined structures. Strain-promoted cycloadditions of the azides of the polymers with functionalized cyclooctynes was highly selective allowing the efficient incorporation of a variety of functional moieties. The oximes functioned as latent dipoles, which upon *in situ* oxidation by hypervalent iodine yielded nitrile oxides as reactive 1,3-dipoles, which could be reacted with modified cyclooctynes to give bi-functionalized polymers. Kinetic measurements demonstrated that pendant nitrile oxides reacted thirty times faster compared to similar cycloadditions with azides and nitrones. The block copolymers served as scaffolds for attachment of hydrophobic and hydrophilic moieties by sequential strain promoted alkyne-azide cycloaddition and strain promoted alkyne-nitrile oxide cycloaddition reactions.

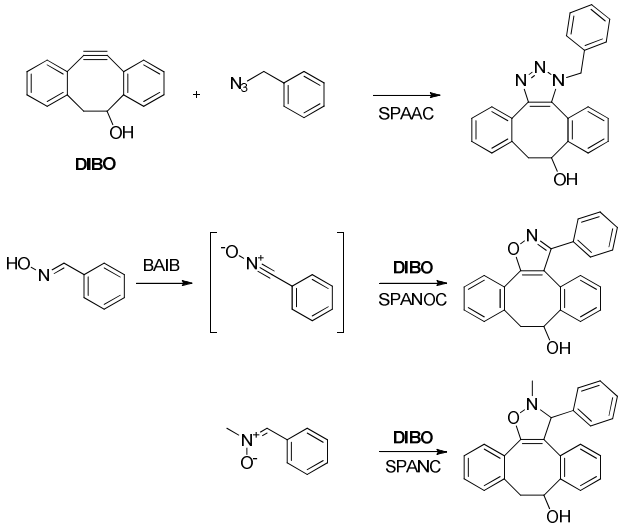
Introduction

Progress in biomedical nanotechnology relies on soft materials with tunable properties and complex yet well-defined architectures.[1] Functional polymers can be prepared either by direct polymerization of monomers bearing a desired functionalization or by post-polymerization modification.[2] The latter, grafting onto approach, has gained popularity due to the development of efficient and chemoselective coupling reactions and polymerization methods that have high functional group tolerance.[3] Grafting onto polymers circumvents problems associated with slow polymerization of complex and bulky monomers resulting in heterogeneity.[4] In addition, post-polymerization modification approaches allow for modular synthesis of libraries of functional polymers from common polymeric precursors thereby offering exciting opportunities to fine tune properties of materials.[5]

Strain-promoted alkyne-azide cycloadditions (SPAAC)[6], which have high functional group tolerance, are relatively fast and do not require toxic metal catalysts, are emerging as an attractive approach for side-[7] and end-functionalization[8] of polymers, creating functional surfaces[9], polymeric networks,[10] and derivatization of dendrimers.[11] Initially, cyclooctynes[12] were employed for these reactions; however their use was limited due to the relatively slow rates of reaction. It has, however, been found that significant increase in the rate of strain-promoted cycloaddition can be accomplished by appending electron-withdrawing groups to the propargylic position of cyclooctyne. For example, difluorinated cyclooctyne (DIFO)[13] reacts with azides approximately sixty-times faster than similar cycloadditions with an unsubstituted cyclooctyne. We have reported that derivatives of 4-dibenzocyclooctynol[14] (DIBO, Scheme 4.1) also react fast with azido-containing compounds and have attractive features such as: easy access to the compound by a simple synthetic approach, non-toxicity and the possibility of straightforward

attachment of a variety of probes.[15] Furthermore, the structure of DIBO is amenable to analog synthesis and derivatives have been introduced that exhibit even higher rates of reaction than the parent compound and other useful properties.[16] In our quest to expand the scope of strain-promoted cycloadditions, we have explored the use of 1,3-dipoles such as nitrile oxides[17] and nitrones[18]. It was found that strain-promoted alkyne-nitrone cycloadditions (SPANC) proceed with rates similar to that of SPAAC, whereas strain-promoted alkyne-nitrile oxide cycloadditions (SPANOC)[19] are sixty times faster. Nitrile oxides can easily be prepared by direct oxidation of the corresponding oximes using hypervalent iodine reagents such as (diacetoxyiodo)benzene (BAIB) (Scheme 4.1). Furthermore, oximes and azides provide an orthogonal pair of functional groups for sequential metal free "click" reactions.

Scheme 4.1 Schematic representation of SPAAC, SPANOC, and SPANC reactions with DIBO



We envisage that polymer functionalization by SPANOC maybe attractive when high rates of reaction are required.[7c] Furthermore, it is to be expected that the use of nitrile oxide and nitrone bearing polymers will expand the types of reactive polymeric scaffolds that are amenable

to modification by strain promoted cycloadditions. It may also alleviate the challenges associated with the synthesis of azido-containing polymers.[20] In addition, oximes can serve as a latent dipole during SPAAC [17] thereby offering possibilities for sequential SPAAC and SPANOC modifications to provide, in controlled manner bi-functional polymers.[21]

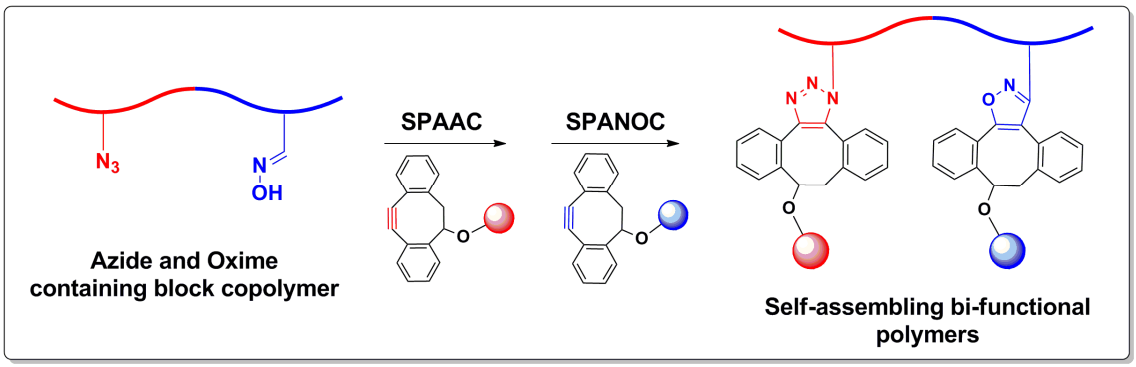


Figure 4.1 Concept of sequential metal-free "click" reactions on azide and oxime-containing polymers for preparation of bi-functional block copolymers

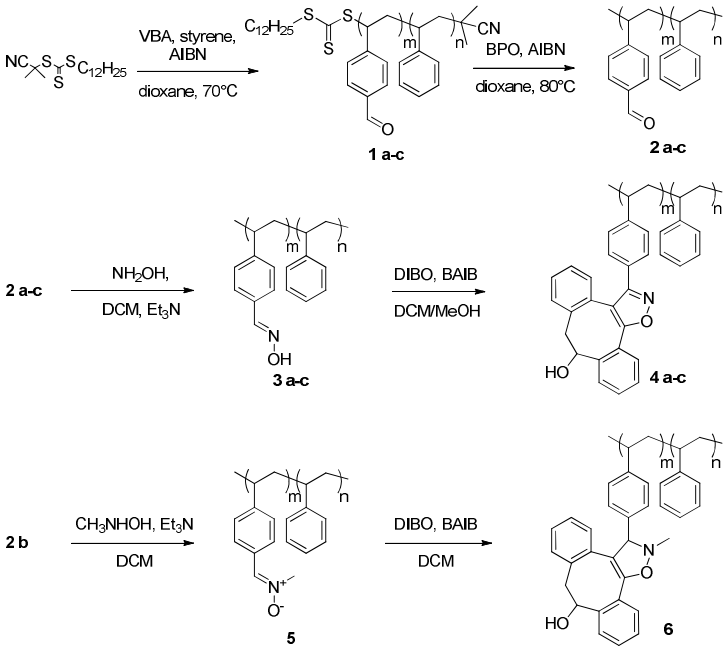
Here, we report the preparation and characterization of polymers containing pendant oxime, nitrone and azide moieties (Figure 4.1). Optimal conditions for cycloadditions of these polymers with DIBO have been studied in detail. RAFT polymerization was utilized to prepare block copolymers containing pendant azides and oximes. Sequential SPAAC/SPANOC reactions with hydrophilic and hydrophobic DIBO-derivatives gave polymers that could self-assemble into well-defined structures.

Results and Discussion

Synthesis of oxime-containing copolymers. First, attention was focused on the development of a procedure for the preparation of nitrone and oxime-bearing polymers. Reactive nitrile oxides can be formed by oxidation of oximes with a hypervalent iodine reagent such as (diacetoxyiodo)benzene (BAIB).[19c] Furthermore, oximes and nitrones can be prepared by reaction of a corresponding aldehyde with hydroxylamine or N-methylhydroxylamine,

respectively. Thus, it was anticipated that 4-vinylbenzaldehyde (VBA)-co-styrene polymers[22], which can be prepared by free radical polymerization without the need for protection of the aldehyde moieties, would be ideal for the preparation of nitrone and nitrile oxide bearing polymers. The VBA-co-styrene polymers **1a-c** were prepared using 2-cyano-2-propyl dodecyl trithiocarbonate as a chain transfer agent and azobisisobutyronitrile (AIBN) as an initiator (Scheme 4.2) *via* RAFT polymerization.

Scheme 4.2 Schematic representation of the synthetic route towards isoxazole bearing polymers **4a-c** and *N*-methyl isoxazole bearing polymer **6**



The VBA monomer was obtained in one step from 1-(chloromethyl)-4-vinylbenzene (VBC) using a Sommelet reaction.[23] Three polymers with varying VBA to styrene ratio were synthesized to establish the proper ratio between functional group density and solubility. Feed ratios of 1:30, 1:20, and 1:5 gave polymers with VBA to styrene ratios of 1:12.3 (**1a**), 1:9.7 (**1b**) and 1:2.7 (**1c**), respectively. The discrepancy between the feed and observed monomer ratios was

expected due to a higher reactivity of VBA comparing to styrene.[22c] The polymerizations were carried out in 1,4-dioxane at 70 ºC for 20 h to achieve 37, 40 and 49 % conversions for polymers **1a**, **1b** and **1c**, respectively. The resulting polymers had monomodal molecular weight distributions and low polydispersity, characteristic for RAFT polymerization. The molecular weights were determined by gel permeation chromatography (GPC) and the results are summarized in Table 4.1.

Table 4.1 Characterization of random copolymers **1-10**

Entry	**M_n(GPC) g/mol[a]**	**M_w(GPC) g/mol[a]**	**PDI (GPC)[a]**
1a	3300	3650	1.10
1b	3400	3700	1.09
1c	4200	4700	1.12
3a	3250	3600	1.11
3b	3350	3700	1.10
3c	4550	5050	1.11
4a	3500	3900	1.11
4b	3800	4150	1.10
4c	4900	5450	1.11
5	2800	3150	1.12
6	3500	3800	1.11
7	3550	3900	1.10
9	3350	3700	1.11
10	3850	4200	1.10

[a]Determined against narrow polystyrene standards at 40 ^{o}C using tetrahydrofuran as the mobile phase.

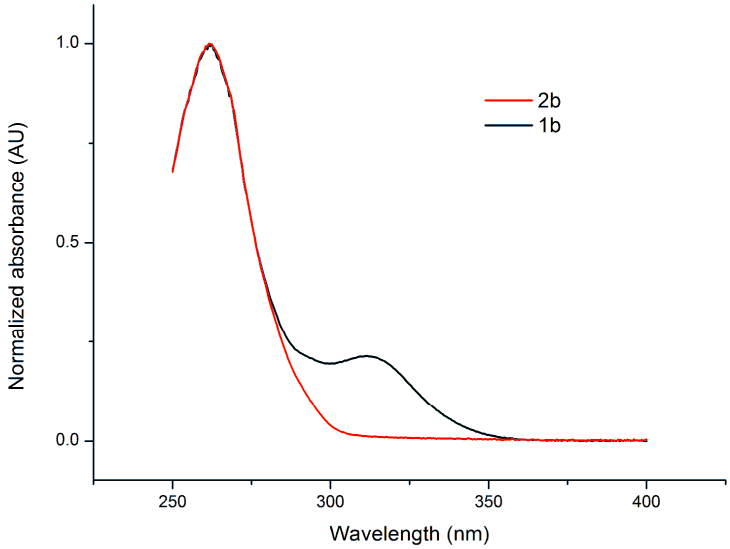


Figure 4.2 UV-Vis spectra of polymers **1b** and **2b** showing the disappearance of trithiocarbonate absorption after free radical fragmentation cleavage. Spectra were taken in $CHCl_3$

The trithiocarbonate RAFT moiety of polymers **1a-c** was cleaved to give **2a-c** using benzoyl peroxide (BPO) and AIBN at 80°C for 10 h.[24] The use of AIBN alone gave incomplete cleavage. In presence of BPO, however, the dodecyltrithiocarbonate cleavage was complete as confirmed by the disappearance of CH_2-S and CH-S proton resonances at 3.2 and 4.8 ppm, respectively in the ^{1}H NMR spectra. The removal of trithiocarbonate was also confirmed by disappearance of the absorption band at 317 nm in UV-Vis spectra (Figure 4.2). A small decrease in molecular weight determined by GPC was also observed for polymers **2a-c**, which is consistent with the removal of a dodecyltrithiocarbonate.

Removal of the trithiocarbonate end groups of polymers **1a-c** was essential because treatment with hydroxylamine to introduce oximes resulted in aminolysis of a RAFT moiety and formation of a free thiol, which dimerized resulting in an increase in molecular weight and PDI. Also, it is known that thiols can react with nitrile oxide thereby compromising the SPANOC

step.[25] Furthermore, aldehydes are incompatible with aminolysis conditions, and thus these conditions could not be employed for modification of the trithiocarbonate end group.[26] We also prepared a polymer similar to **1** using 4-vinylbenzaldehyde diethyl acetal as a monomer and employed aminolysis/thio-Michael addition for removal of the RAFT moiety.[27] This route was, however, lengthier due to the need of acetal protection-deprotection steps. Also diethyl acetal moieties were rather labile and polymers bearing this functional group could not be stored for a prolonged period of time. Polymers **2a-c** were treated with hydroxylamine and triethylamine in dichloromethane (DCM) to give oxime containing polymers **3a-c**. The quantitative conversion of the aldehyde moieties to oximes was confirmed by the disappearance of the aldehyde (CHO) signal at 9.9 ppm and appearance of oxime (CHN) singlet at 8.08 ppm in ^{1}H NMR (Figure 4.4). In addition, disappearance of a carbonyl stretch in IR spectra at ca. 1700 cm^{-1} confirmed the completion of the reaction (Figure 4.5).

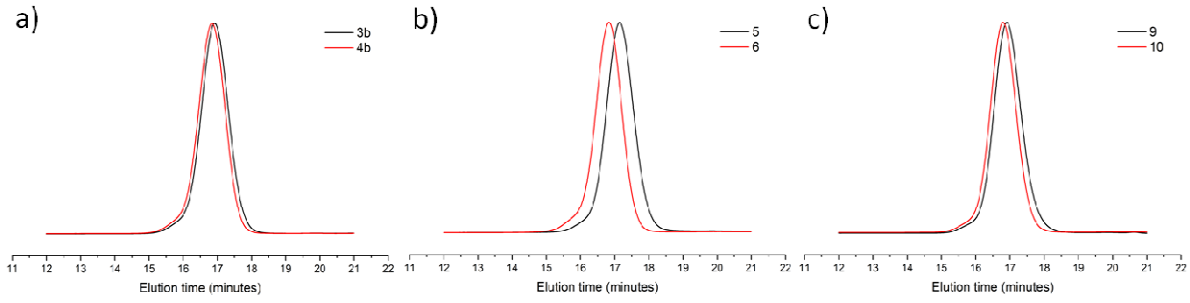


Figure 4.3 GPC traces of polymers before and after reaction with DIBO. a) **3b** and respective isoxazole–bearing polymer **4b**. b) **5** and respective N-methyl isoxazole–bearing polymer **6**. d) **9** and respective triazole–bearing polymer **10**

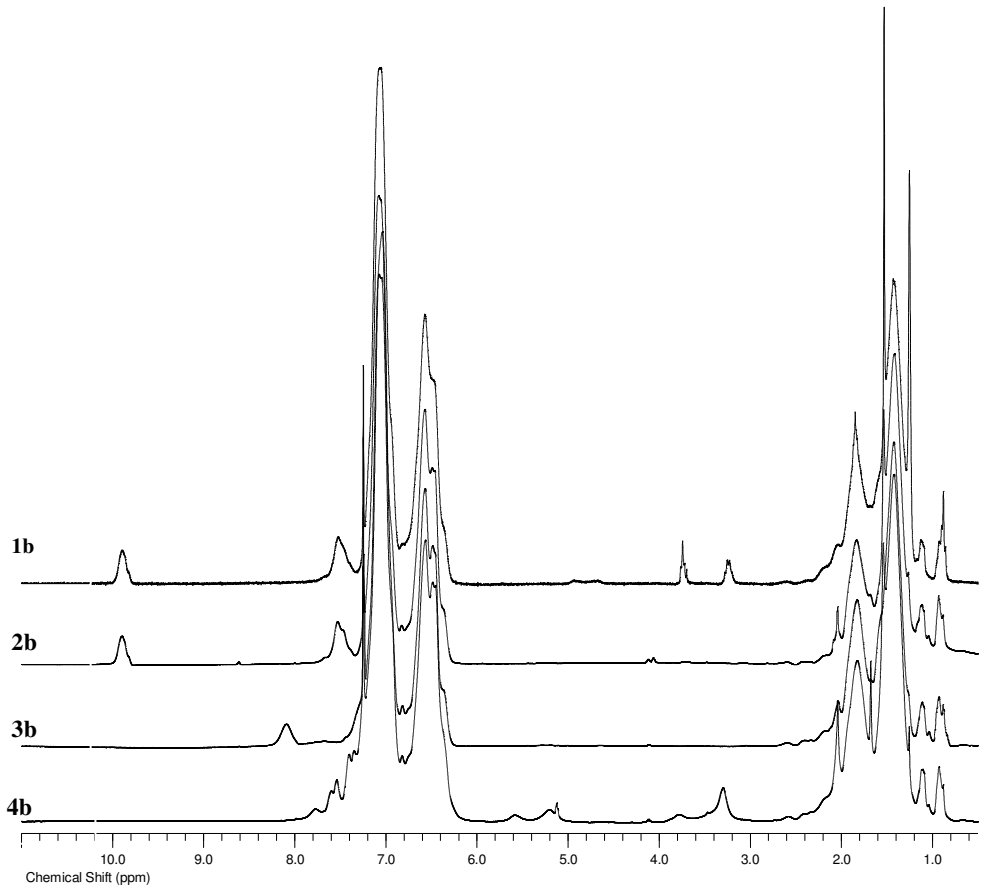


Figure 4.4 ^{1}H NMR spectra of polymers **1b-4b**

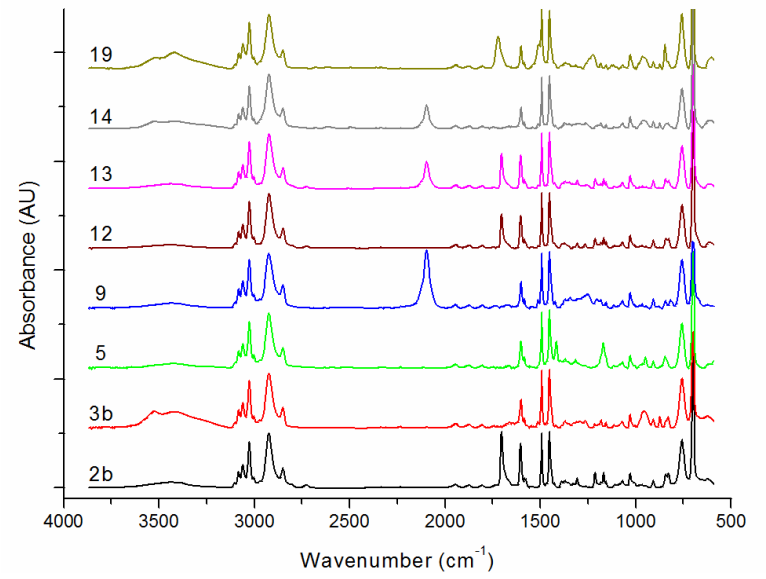


Figure 4.5 Representative IR spectra of functional copolymers **2b, 3b, 5, 9, 12, 13, 14, and 19**

Although the conversion was quantitative for all three polymers, it was found that solubility of oxime-rich polymer **3c** in organic solvents was inferior to polymers with lower oxime content. Therefore, for the subsequent experiments polymer **3b** was used having a VBA:styrene ratio of 1:9.

Nitrone-bearing polymer **5** was conveniently prepared by treatment of **2b** with *N*-methylhydroxylamine hydrochloride and triethylamine in DCM (Scheme 4.2).[28] Quantitative nitrone formation was evident from the disappearance of the aldehyde (CHO) signal in ^{1}H NMR spectra at 9.90 ppm and appearance of distinct (CH_{ar}) and CH_3N signals at 7.9 and 3.85 ppm, respectively and also by disappearance of a carbonyl stretch signal in the IR spectra (Figures 4.2, 4.5).

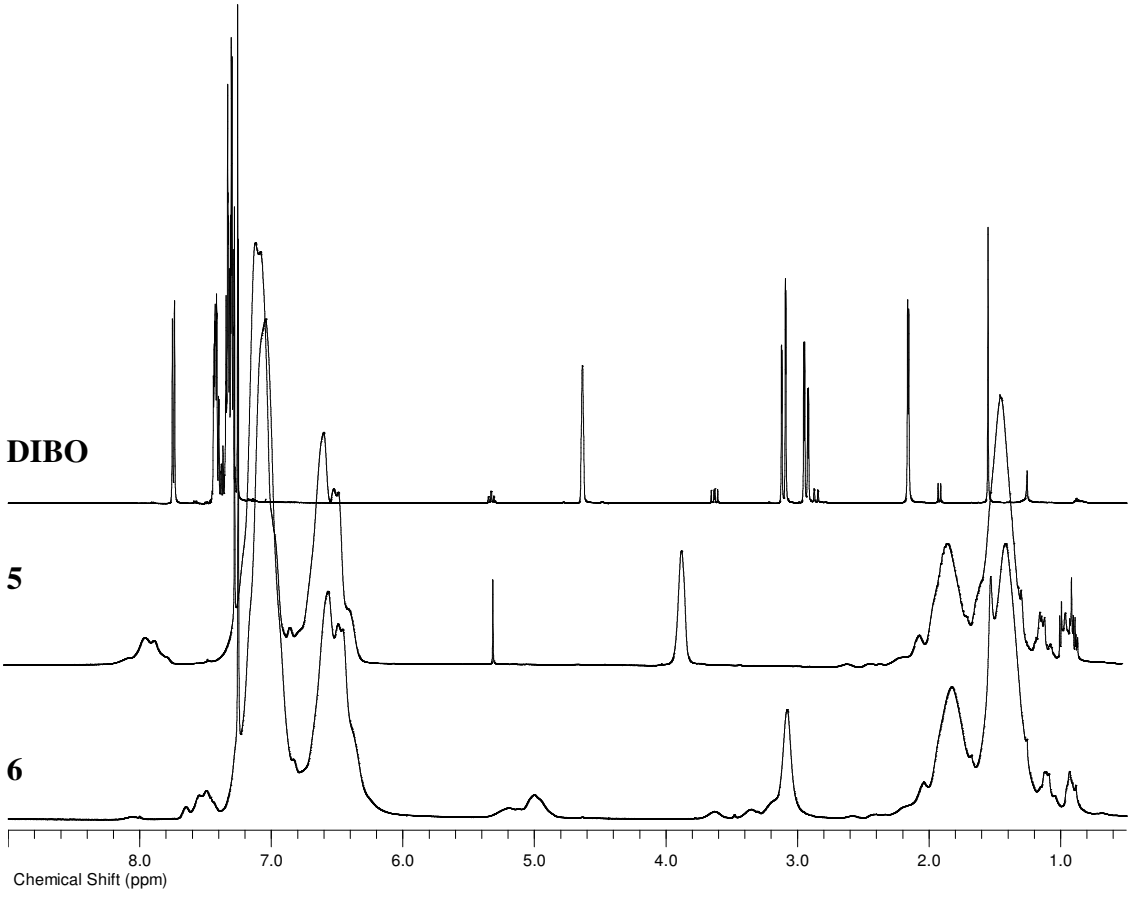


Figure 4.6 ^{1}H NMR spectra of DIBO and polymers **5** and **6**

Finally, a copolymer of styrene and 1-(chloromethyl)-4-vinylbenzene (VBC) (**7**) was prepared which served as the precursor for the preparation of azido-functionalized polymer **9** (Scheme 4.3). The polymerization was carried out in 1,4-dioxane at 70°C using AIBN as initiator and 2-cyano-2-propyl dodecyl trithiocarbonate as a chain transfer agent to achieve a conversion of 40% in 20 h. The VBC monomer has a slightly higher reactivity than styrene and therefore a feed ratio of 1:10 VBC to styrene resulted in polymer **7** having 1:7.6 VBC to styrene ratio. The VBC-*co*-styrene polymer **7** had a monomodal molecular weight distribution and a narrow PDI (GPC) (Table 4.1). Next, the RAFT moiety of polymer **7** was cleaved by the treatment with BPO and AIBN at 80°C in dioxane for 10 h. The RAFT cleavage was complete as confirmed by NMR and UV-Vis spectroscopy. Comparison of GPC chromatograms showed a slight reduction of a molecular weight of polymer **8** (M_n = 3400 g/mol, GPC) comparing to **7** (M_n = 3550 g/mol, GPC) corroborating the dodecyltrithiocarbonate cleavage. The chlorides of polymer **8** were quantitatively displaced by NaN_3 in DMF to give polymer **9** as shown by IR (Figure 4.5) and NMR spectroscopy (Figure 4.7). An attempt to directly polymerize an azido-containing monomer 1-(azidomethyl)-4-vinylbenzene (AzMVB) at 70 °C resulted in a polymer having high molecular weight shoulders probably due to nitrene formation and subsequent crosslinking or cycloaddition of azide to a double bond of styrenic monomers.[20] Also, treatment of the polymer **7** with sodium azide resulted in partial cleavage of RAFT moiety resulting in an increase in PDI, probably due to the formation of disulfides.

Optimization of reaction conditions for polymer modification by SPANOC. Nitrile oxides are reactive species that can dimerize to form furoxans.[29] For example, benzohydroxamoyl chloride has been reported to dimerize to give bis-phenyl-3,4-furoxan when treated with triethylamine at room temperature.[30]

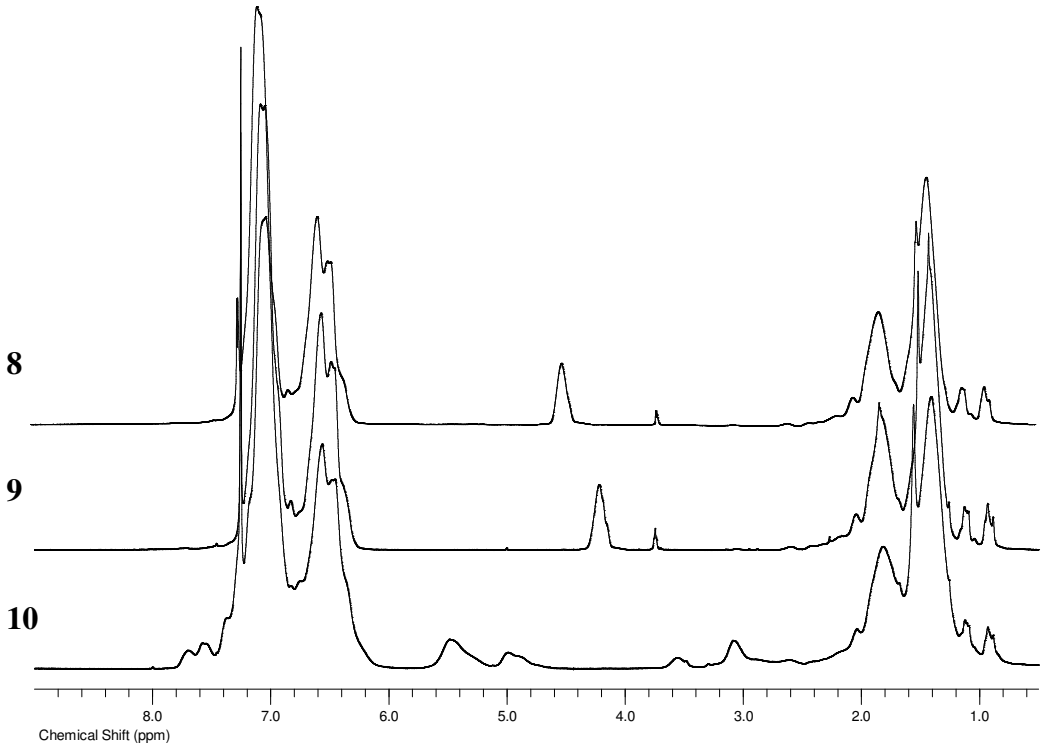
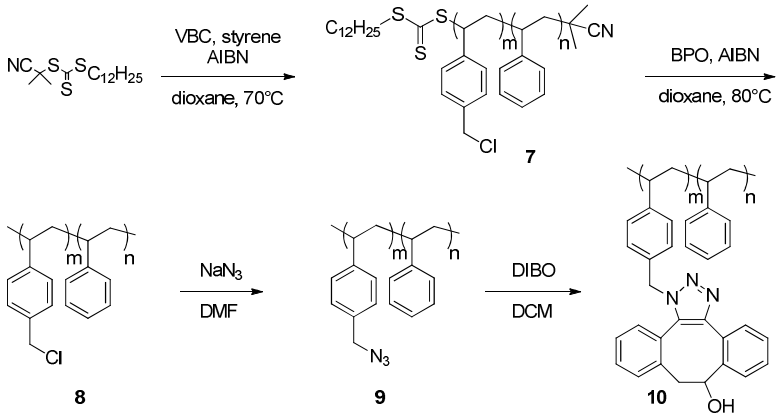


Figure 4.7 ^{1}H NMR spectra of DIBO and polymers **8, 9** and **10**

Scheme 4.3 Schematic representation of the synthetic route towards triazole bearing polymer **10**



On contrary, BAIB mediated oxidization of benzaldoxime to give the corresponding nitrile oxide in absence of dipolarophiles or nucleophiles, gave *N*-acetoxy benzamide as the major product and not the dimerization adduct.[31] When polymer **3a** was treated with BAIB in absence of DIBO, a high molecular weight shoulder was observed in the GPC chromatogram of the resulting product (Figure 8). This observation indicates that in absence of a dipolarophiles, polymeric nitrile oxides can undergo intermolecular dimerization. The crosslinking was observed during conversion of **3a** even at low concentrations (2 mM) in DCM (Figure 4.8).

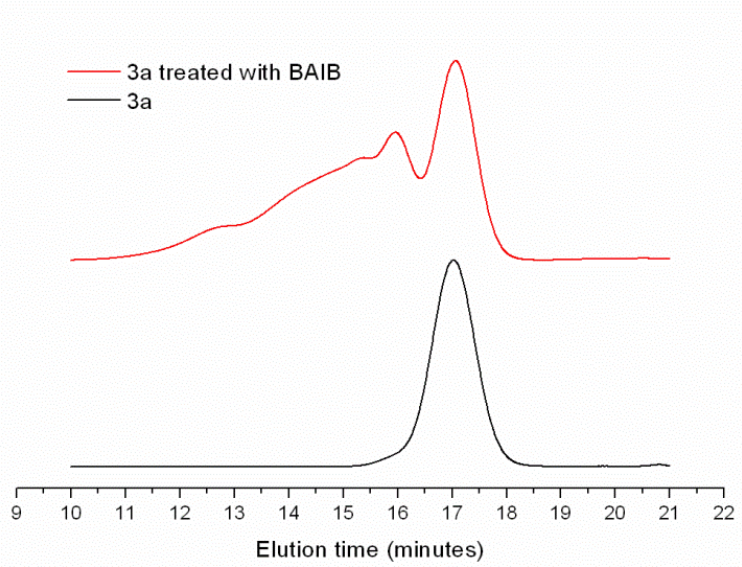


Figure 4.8 GPC chromatograms of polymers **3a** and a partially crosslinked polymer formed as a result of treatment of **3a** with BAIB in absence of DIBO

However, no cross linking was observed when DIBO (3 eq) was premixed with polymer **3a** in a mixture of methanol (MeOH) and DCM (4/1, v/v) followed by the drop wise addition of a solution of BAIB. The formation of isoxazole **4a** was confirmed by the disappearance ^{1}H NMR C*H*N signal of oxime at 8.08 ppm and appearance of new broad signals of isoxazole at 3.12-3.85 (CH_2CH) and 5.14-5.58 (C*H*OH) ppm (Figure 4.4). The extent of the modification was approximately 90%, based on integration of these peaks and aldehyde signal of polymer **1a**.

Similarly, treatment of polymers **3b** and **3c** with DIBO and BAIB gave, after purification by precipitation from cold MeOH, monodisperse polymers without a sign of intermolecular cross linking. For polymer **4a**, the level of modification was 98% and for polymer **4c** 81%. GPC traces of all three isoxazole bearing polymers **4a-c** showed a slight decrease in elution time indicating an increase of the molecular weight as a result of successful pendant modification (Figure 4.3). Similarly, treatment of polymer **5** with small excess of DIBO in DCM gave *N*-methyl isoxazole bearing polymer **6**. The quantitative conversion of nitrone groups was evident from the ^{1}H NMR spectrum, which showed a disappearance of CH_{ar} and CH_3N signals of the nitrone at 8.0 and 3.9 ppm, respectively and appearance of new broad signals of *N*-methyl isoxazole at 3.0-3.8 (C*H*$_2$CH), 4.9-5.2 (C*H*OH), 3.1 ppm (CH_3N), and 4.9-5.2 ppm (CHN) (Figure 4.6). Interestingly, GPC of the polymer **6** showed a significant increase in hydrodynamic volume compared to precursor polymer **5**. We hypothesize that due to the conversion of polar nitrones into isoxazoles of a lower polarity; polymer **6** is better solvated in tetrahydrofuran (THF) and therefore adopts an extended conformation. Finally, a triazole bearing polymer **10** was obtained by treatment of azido containing polymer **9** with DIBO in DCM. The complete consumption of the azide groups was confirmed by IR (Figure 4.5) and ^{1}H NMR spectroscopies (Figure 4.7). GPC of the polymer **10** shows a modest increase in molecular weight due to pendant modification (Figure 4.3).

Reaction kinetic measurements of SPAAC, SPANC and SPANOC. Having established that the 1,3-dipoles of polymers **3a-c**, **5** and **9** can react cleanly with DIBO, attention was focused on determining the second order rate constants of the cycloadditions. For this purpose, polymers **3b**, **5** and **9** were chosen because they have a similar molecular weights and functional group densities. The rate constants were determined following the consumption of

DIBO by ^{1}H NMR spectrometry using $CDCl_3$ as a solvent (See Experimental Section for details). It was found that nitrile oxides are the most reactive species, which is in accordance with our previous observations using low molecular weight compounds (Figure 4.9).[17]

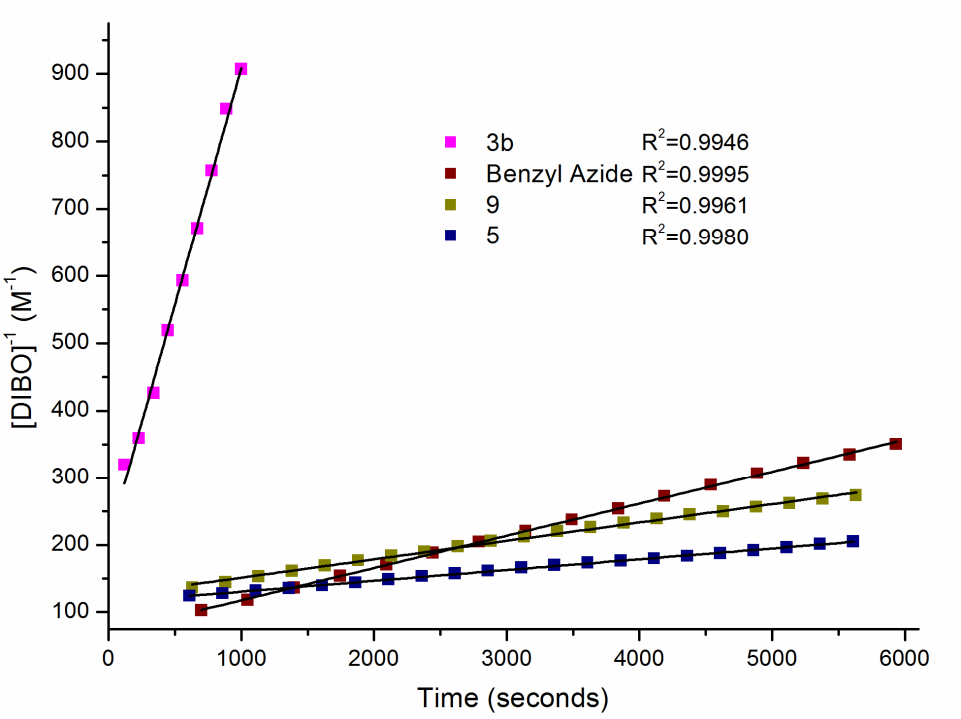


Figure 4.9 Representative kinetic plots for cycloadditions of benzyl azide (20 mM), polymer **3b** (6.7 mM), **5** (10 mM) and **9** (10 mM) with DIBO

The second order rate constant for the SPANOC cycloaddition of polymer **3b** with DIBO was found to be 0.773±0.196 $M^{-1}s^{-1}$ (Table 4.2). The azido-containing polymer **9** and nitrone bearing polymer **5** exhibited similar reactivities with second order rate constants of 0.027±0.002 $M^{-1}s^{-1}$ and 0.016±0.01 $M^{-1}s^{-1}$, respectively. The data shows that that SPANOC is approximately thirty times faster than the SPAAC. Interestingly, the rate constant of cycloaddition of azido-

containing polymer **9** with DIBO was almost two times smaller than that of benzyl azide, which is probably a consequence of steric hindrance caused by the polymeric nature of the 1,3-dipoles.

Table 4.2 Second order DIBO cycloaddition rate constants for benzyl azide and polymers **3b, 5, 9** obtained from three independent measurements

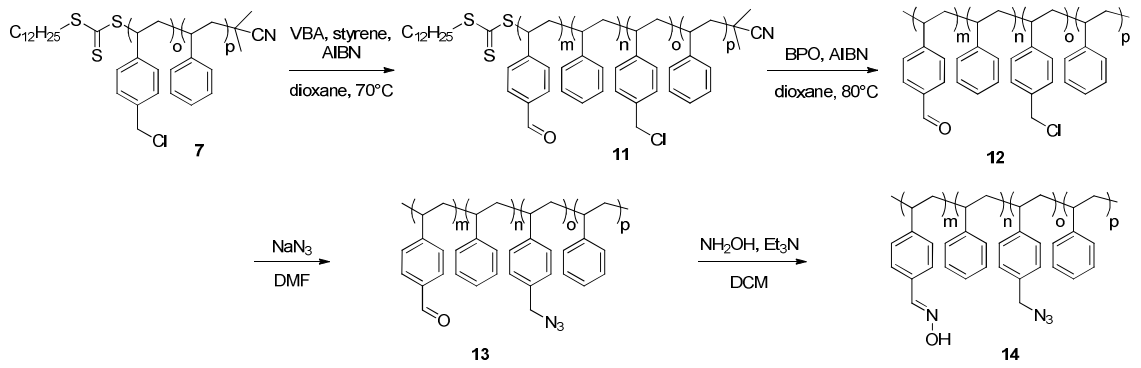
Dipole	**Second Order Rate constants ($M^{-1}s^{-1}$)[a]**			
	1	2	3	Average
Benzyl azide	0.052	0.037	0.048	0.046±0.008
3b	0.995	0.702	0.623	0.773±0.196
5	0.015	0.016	0.016	0.016±0.001
9	0.030	0.027	0.025	0.027±0.002

[a] Measurements were performed in $CDCl_3$ at 25ºC.

Synthesis of bi-functional block copolymers containing azide and oxime moieties. Previously, we demonstrated that oximes can serve as latent 1,3-dipoles making it possible to perform sequential SPAAC/SPANOC reactions. Therefore, it was expected that copolymers having azide and oxime groups would allow bi-functionalization in a controlled manner.[17] It was envisaged that due to the living nature of RAFT polymerization, VBC-containing polymer **7** can be employed as a macro chain transfer agent for further polymerizing with 4-vinylbenzaldehyde to give block copolymers having chloride and aldehyde moieties in separate blocks (Scheme 4.4). The chlorides of the resulting polymers can then be displaced with NaN_3 and the aldehydes reacted with hydroxylamine to give oximes. The azides can then be reacted with functionalized

DIBO, followed by oxidation of the oximes to nitrile oxides which in turn can be reacted with another DIBO derivative having another functional group.

Scheme 4.4 Schematic representation of the synthetic route towards bi-functional polymer **14**



Synthesis of bi-functional block copolymer **11** was achieved by copolymerizing VBA and styrene in 1,4-dioxane for 20 h at 70°C using AIBN as initiator and polymer **7** as a macro-CTA. The molar ratio [AIBN][CTA][VBA][Styrene] in the feed was 0.001:0.01:0.05:1 in order to achieve the block length and functional density similar to that of polymer **1b**. According to GPC, the molecular weight of resulting polymer **11** (7700 g/mol) was twice as large compared to precursor polymer **7**, demonstrating successful chain extension (Table 4.3).

Table 4.3 Characterization data of block copolymers **11, 14, 19, 20, 21 a-c, 22 a-c**

Entry	**M_n(GPC)[a] g/mol**	**M_w(GPC)[a] g/mol**	**PDI(GPC)[a]**
11	7700	8800	1.15
14	7100	7950	1.12
19	7850	8850	1.13
20	10900	13450	1.23

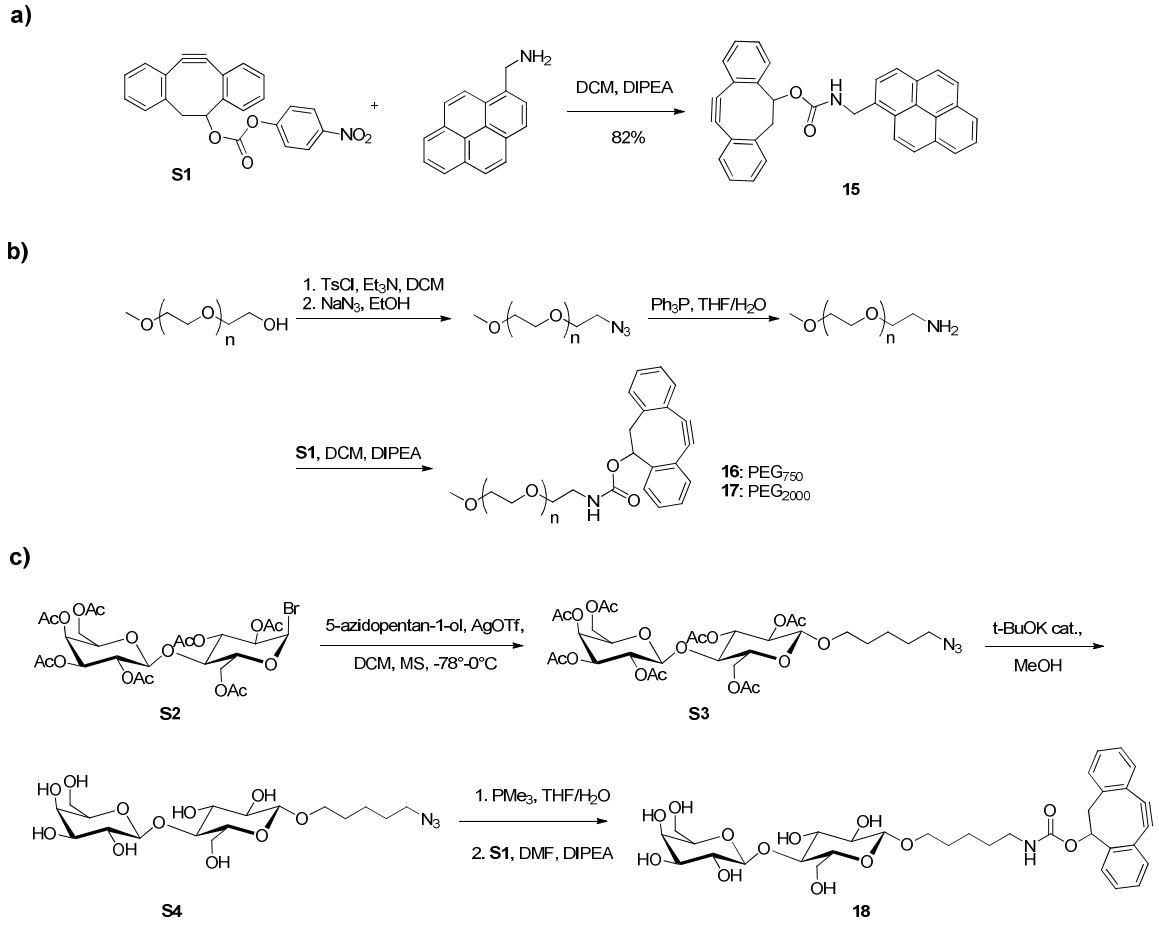
21a	9450	10800	1.14
21b	12150	13550	1.12
21c	6850	7950	1.16
22a	10550	12200	1.16
22b	15200	17700	1.16
22c	7250	8600	1.18

[a]Determined against narrow polystyrene standards at 40 ^{o}C using THF as the mobile phase.

Polymer **11** has approximately equal numbers of chloride and aldehyde groups (approximately four of each per polymer chain) as determined by comparing integral areas of CH_2Cl (4.50 ppm) and CHO (9.90 ppm) signals in the ^{1}H NMR spectrum. The RAFT moiety was cleaved using conditions described for compounds **2** and **8** to give block copolymer **12**. The chlorides were quantitatively converted into azides by using NaN_3 in DMF as confirmed by IR (Figure 4.5) and ^{1}H NMR. Finally, treatment of polymer **13** with hydroxylamine afforded the target bi-functional block copolymer **14** bearing azide and oxime groups in distinct blocks.

Polymers, such as **14**, can be modified with DIBO derivatized with a wide variety of moieties. Furthermore, this block copolymer architecture and the possibility to sequentially attach hydrophilic and hydrophobic compounds, offers opportunities to prepare materials that can self-assemble in aqueous medium. To test this hypothesis, four different DIBO derivatives were prepared having hydrophobic pyrene (**15**), hydrophilic polyethyleneglycol (PEG) moieties of different molecular weights (**16** and **17**) and a disaccharide, lactose (**18**) (Scheme 4.5).

Scheme 4.5 Synthesis of DIBO conjugates **15-18**

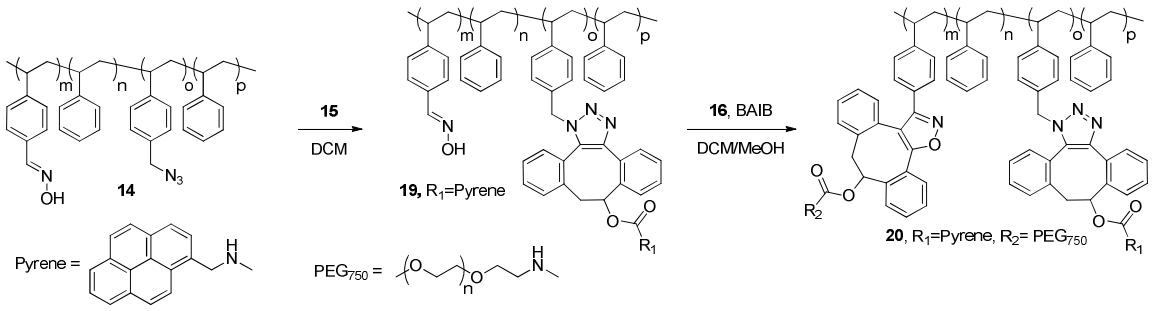


Derivative **15** was prepared by coupling of 1-pyrenemethylamine hydrochloride with carbonic acid 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl ester[15a] (S1) in presence of *N*,*N*-diisopropylethylamine. DIBO-PEG derivatives were prepared by first tosylation of respective poly(ethylene glycol) methyl ethers and subsequent introduction of azide groups by treatment with sodium azide. Staudinger reduction of azides gave free amines which could be reacted with **S1** giving derivatives **16** and **17**. Synthesis of DIBO-Lactose derivative **18** started with peracetylated lactosyl bromide **S2** which was glycosylated with 5-azidopentanol[32] using silver trifluoromethanesulfonate as promoter. The resulting compound was deacetylated

under basic conditions and after reduction of the azide coupled to **S1** to give DIBO-Lactose derivative **18**.

SPAAC coupling of block copolymer **14** with hydrophobic DIBO-pyrene **15** at room temperature in DCM for 18 h afforded polymer **19** (Scheme 4.6). Quantitative formation of triazoles was confirmed by the disappearance of methylene signals at 4.21 ppm in the ^{1}H NMR spectra and the disappearance of azide signal in IR spectra (Figure 4.5). Also, the NMR data indicated that the oxime groups had remained intact during the transformation. Next, a SPANOC reaction was carried out between polymer **19** and DIBO-PEG$_{750}$ (**16**) in presence of BAIB in a mixture of MeOH:DCM (1:5 v/v) for 4 h.

Scheme 4.6 Schematic representation of the synthetic route towards amphiphilic polymer **20**



Polymer **20** was isolated by precipitation with cold MeOH and the resulting product was characterized by ^{1}H NMR spectroscopy, IR spectroscopy and GPC. Although the SPANOC reaction led to efficient grafting of hydrophilic PEG$_{750}$ moieties on the polymer backbone, the resulting constructs had relatively high PDI and exhibited a high molecular weight shoulder in GPC chromatograms (Figure 4.10b). Due to the fact that no apparent crosslinking of nitrile oxide moieties was observed for polymers **4a-c**, having similar or higher functional density as polymer

19, we hypothesized that stacking of the pyrene moieties during SPANOC-mediated modification resulted in polymer aggregation, which in turn induced cross-linking observed by GPC. Indeed, the fluorescence emission spectrum of polymer **19** in $CHCl_3$ showed a strong band with maxima at 525 nm (Figure 4.10a), supporting stacking of pyrene moieties and formation of excimers. The excimer emission band persisted even in highly diluted solutions of polymer **19** (up to 0.01 μg/mL in $CHCl_3$, data not shown).

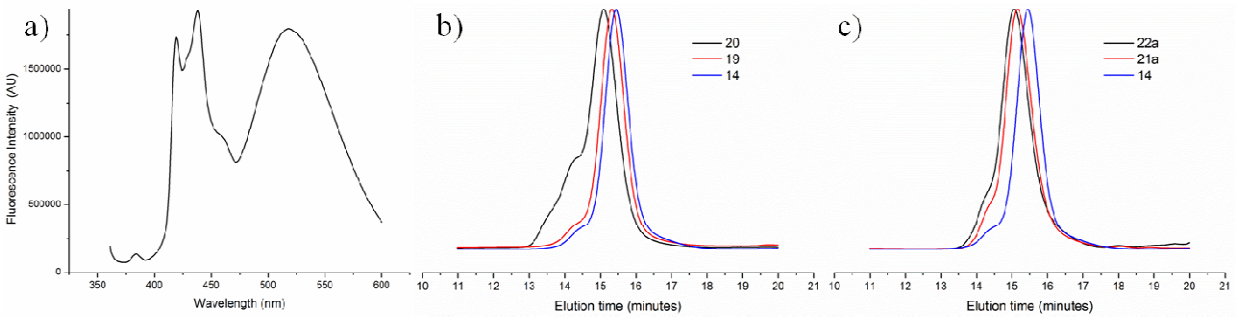
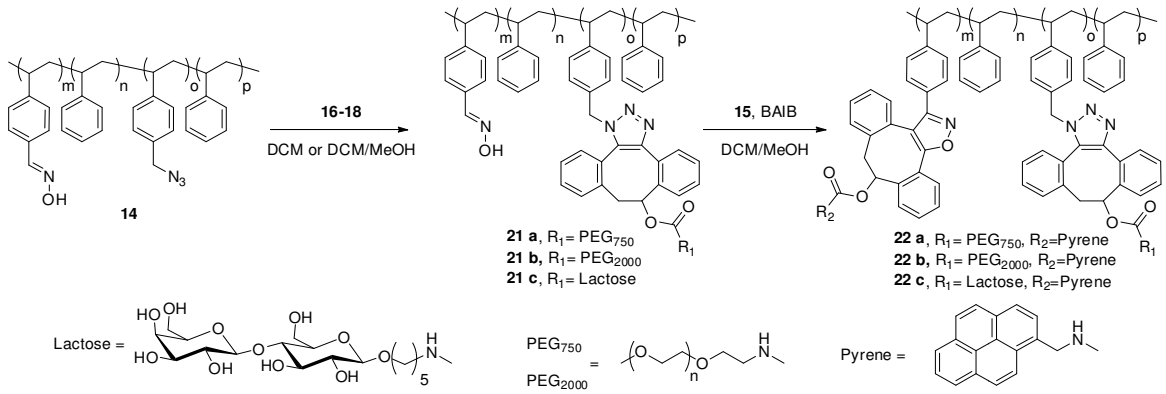


Figure 4.10 a) Fluorescence spectra of polymer **19** in $CHCl_3$ (1 μg/mL) excitation at 310 nm. b) GPC traces of polymers **14, 19** and **20**. c) GPC traces of polymers **14, 21a** and **22a**

Scheme 4.7 Schematic representation of the synthetic route towards amphiphilic polymers **22a-22c**



To avoid cross-linking, the order of attachment of the hydrophilic and hydrophobic moieties was changed (Scheme 4.7). Thus, polymer **14** was first derivatized with PEG_{750} (**16**) *via* SPAAC to give polymer **21a**, which was then reacted with DIBO-Pyrene conjugate (**15**) in presence of BAIB to provide bi-functional polymer **22a**. Fortunately, the high molecular weight shoulder in the GPC chromatogram of polymer **22a** was significantly reduced (Figure 4.10c). Polydispersity values obtained from GPC further supported a better control over functionalization; polymer **22a** had PDI=1.16 (M_n=10550, M_w=12200 g/mol) whereas polymer **21** had PDI=1.23. In a similar fashion, graft copolymers with hydrophilic PEG_{2000} and unprotected lactose side chains were prepared from polymer **14** and DIBO-conjugates **17** and **18**. The solvent of choice for SPAAC-mediated coupling of polymer **14** and PEG derivative **17** was DCM. The excess of DIBO-PEG reagent **17** was removed by treatment with azide resin (see experimental section for preparation details) for 16 h (overnight) to give pure polymer **21b**. Grafting of lactose onto polymer **14** required the use of 1:1 MeOH/DCM mixture as a solvent. The resulting polymer **21c** was purified by precipitation into cold MeOH, and grafting efficiency was 82% as determined from ^{1}H NMR. Interestingly, the hydrodynamic radius of a lactose-modified polymer was significantly reduced according to GPC, probably due to the collapsed conformation of the lactose-containing polymer in THF used for GPC. Finally, oxime bearing polymers **21b** and **21c** were reacted with DIBO-Pyrene conjugate (**15**) in presence of BAIB in MeOH/DCM (1:5) mixture for 4 h to provide bi-functional polymers **22b,c.** Polymers **22a-c** were isolated by precipitation into cold Et_2O/Hexanes (1:2 v/v) mixture twice to remove excess of reagents. DIBO derivatives **15-18**, have a strong absorbance at 310 nm, which was not observed in the UV spectra of polymers **22 a-c** indicating complete removal of the excess of DIBO conjugates (Figure 4.11).

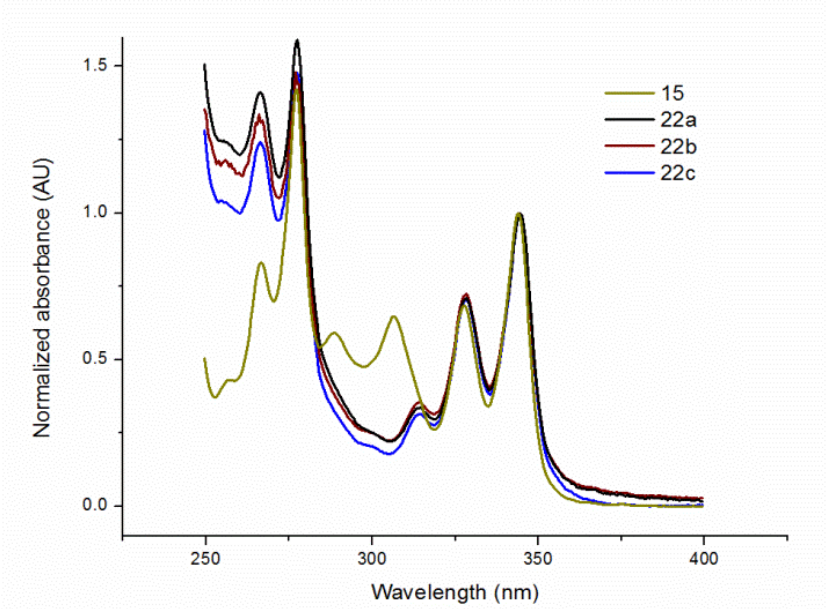


Figure 4.11 UV-Vis spectra of polymers **22a-c** and DIBO-Pyrene **15** in $CHCl_3$

Dynamic Light Scattering and Transmission Electron Microscopy study of polymer self-assemblies. Following the successful synthesis of comb shaped block copolymers with hydrophilic and hydrophobic pendant groups, the self-assembly properties of polymers **22a-c** were studied. The polymers were dissolved in THF (10 mg/ml) and the resulting solution slowly added to distilled water while stirring to reach a final concentration of 1 mg/ml. After stirring for 2 h to allow the organic solvent to evaporate, the resulting solutions were filtered through 0.8 µm filter. Dynamic light scattering (DLS) of the resulting aqueous solutions showed formation of self-assembled materials only for polymers **22a** and **22c**. According to DLS polymer **22a** formed nanoparticles with mean diameter of 78.0 nm and polydispersity of 0.112. Polymer **22c** self-assembled into larger particles with mean diameter of 122.0 nm with polydispersity of 0.147.

To confirm the DLS results, the self-assembled polymers **22 a-c** were examined by transmission electron microscopy (TEM). In support of DLS data, polymer **22a** having pendant

PEG_{750} moieties assembled into polydisperse spherical structures having a size range of 20 to 150 nm (Figure 4.12a).

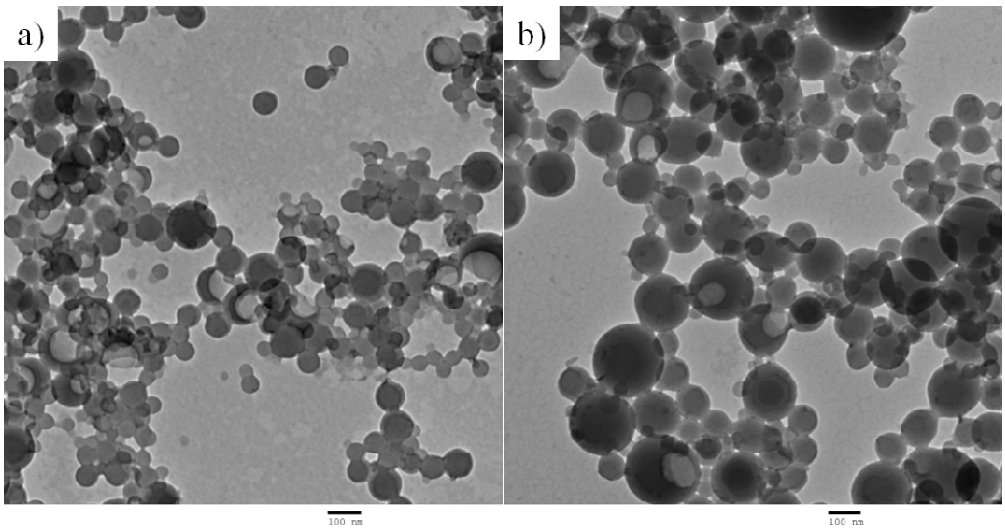


Figure 4.12 TEM images of drop casted aqueous solutions of polymers, stained with uranyl acetate. a) polymer **22a**, b) polymer **22c**. Scale bar is 100 nm

Polymer **22 b** did not form well-defined nanoparticles, instead rod and sheet-like structures were observed by TEM. We hypothesize that the hydrophilic segment of this polymer is too large to form stable nanoparticles. On the other hand, polymer **22a** has suitable hydrophilic-hydrophobic balance to form stable assemblies in aqueous solutions. Polymer **22c** with grafted Lactose moieties also demonstrated robust self-assembly forming larger nanoparticles with sizes of 60 - 300 nm according to DLS and TEM (Figure 4.12b).

Conclusion

Multi-functional polymers containing orthogonal pendant functionalities for postpolymerization modification offer a versatile scaffold for creating new materials with controlled properties. In particular, hetero-bi-functional block copolymers with distinct reactive groups in each block can be used to prepare materials self-assembling in solution or in bulk to be used in drug delivery, nanolithography and preparation of nanomembranes.[33] There are, however, few reported methods for the preparation of such polymeric scaffolds. Maynard and coworkers employed RAFT polymerization to prepare a bi-functional block copolymer that have activated ester and aldehyde groups amenable for post-polymerization modification.[21b] Theato and coworkers also used activated esters of different reactivity for sequential modification of polymeric backbone with various amines.[21e] Finally, Hawker and coworkers designed polylactide-based block copolymer for orthogonal CuAAC and thiol-ene modifications.[21h] The main challenges for the construction of such polymers include i) a need for facile introduction of reactive groups onto polymer backbone or tolerance to polymerization conditions; ii) high selectivity and functional group tolerance for post-polymerization modification; and iii) limiting the number of reactive species required for post-polymerization modification. The SPANOC reaction developed by us satisfies these requirements and in particular i) the oxime is easily introduced *via* an aldehyde precursor; ii) oximes are stable during SPAAC and therefore two reactions can be performed in a sequential manner; and iii) both nitrile oxides and azides add to cyclooctynes such as DIBO thereby reducing the number of derivatives required for post-polymerization multi-functionalization. In this work we demonstrated that block copolymers containing both azide and oxime moieties in segregated blocks can be prepared and derivatized with hydrophilic and hydrophobic moieties to give amphiphilic bottle-brush copolymers. The

modular nature of the synthetic approach makes it possible to determine in a facile manner the optimal hydrophilic-hydrophobic balance for self-assembly of the resulting polymers in aqueous medium. The expansion of sequential SPAAC/SPANOC reactions in the area of polymeric scaffolds greatly facilitates the design and assembly of intricate polymeric structures for applications in material science and biomaterials. Furthermore, the kinetic study revealed that the rate of SPANOC cycloaddition was 30 times greater than that of SPAAC. Nitrones were shown to have similar to azides reactivity towards DIBO. This is the first time the SPANOC and SPANC were employed for post-polymerization modification of polymers. Future studies to extend this chemistry to thin films are underway.

Experimental section

All reagents were purchased from Sigma-Aldrich and used as received unless stated otherwise. Anhydrous tetrahydrofuran (THF), methanol (MeOH) and *N,N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich and EMD. Compounds **S1** and **S2** were prepared following previously reported procedures.[15a, 34] Dichloromethane was distilled over calcium hydride. Styrene and 4-vinylbenzyl chloride were washed with 1N NaOH, followed by water to remove inhibitors, dried over $MgSO_4$ and then purified by vacuum distillation over calcium hydride. AIBN was recrystallized from MeOH twice prior to use. Azido resin was synthesized by reacting Merrifield's resin (3.5-4.5 mmol/g) with excess of sodium azide in DMF overnight at 80°C and subsequent thorough washing with MeOH and dichloromethane (DCM). Reactions were performed at room temperature (20-22°C), unless stated otherwise. Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed EMD silica gel 60 (F254) plates, visualized using UV light (254 nm) and potassium permanganate and cerium molybdate dips as appropriate. Flash chromatography was carried out using silica gel G60

(SiliCycle, 60-200μm 60 Å) as the stationary phase. The NMR spectra were recorded on Varian Mercury (300, 500 MHz) spectrometers at 25˚C. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS, spectra are referenced by solvent signals. Coupling constants (J) are measured in Hertz (Hz) and are unadjusted. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, m – multiplet, br – broad. Various 2D NMR techniques (COSY, HSQC) were used to establish the structures and to assign the signals. ^{1}H NMR-based M_n of dodecyltrithiocarbonate-terminated polymers was calculated by comparing the integral areas under CH_{ar}, CHO, CH_2Cl peaks of repeating units with average integrals of CH-S, CH_2-S and CH_3 signals of ω-chain end. The weight of CTA (345 g/mol) was then added to the sum of weights of repeating units. NMR-based M_n was used to calculate quantities of reagents for dodecyl trithiocarbonate cleavage. For side chain transformations the quantities of reagents were calculated based on ratio of repeating units obtained from ^{1}H NMR. Mass spectra were obtained using MALDI-ToF instruments (ABISciex 5800 MALDI-TOF-TOF) with 2,5-dihydroxybenzoic acid or dithranol as a matrix. Positive reflector mode was used unless stated otherwise. Monoisotopic masses are provided unless stated otherwise. Gel permeation chromatography (GPC) analyses were performed in on Shimadzu LC-20AD liquid chromatography instrument, equipped with RI detector. Two Waters Styragel columns (HR3 and HR4) were placed in series. THF was used as eluent at 1ml/min flow rate; the column oven was set to 40ºC. Molecular weights were calculated against polystyrene standards. IR spectra were acquired on Thermo-Nicolet 6700 FTIR Spectrometer in KBr pellets.

Kinetics measurements were performed by setting up ^{1}H NMR array experiment in $CDCl_3$ at 25ºC. The preacquisition delay was set to 10 s for polymer **3b**, and 150 s for polymers

6, 9 and benzyl azide. The concentration of DIBO at any given time was calculated from conversion which in turn was obtained by integrating aliphatic CH and CH_2 signals of DIBO and CH signals of triazole or isoxazole. The number of scans was 16 for benzyl azide and 32 for polymers. The concentrations of reactants were 0.02 M for benzyl azide, 0.01 M for polymers **6, 9** and 0.0067 M for polymer **3b**. Polymer **3b** was premixed with 2 equivalents of (diacetoxyiodo)benzene (BAIB) for 11 min before addition of DIBO. All experiments were performed in triplicates. The second order rate constants ($1/[DIBO]=1/[DIBO]_0+kt$) were determined from the slope of the plot of 1/[DIBO] vs time.

TEM observations were made using a Philips/FEI Tecnai 20 instrument operating at an accelerating voltage of 200 kV. Dilute solutions of the polymeric nanoparticles in water (1 mg/mL) were deposited in copper grids coated with carbon (Electron Microscopy Science (EMS), Hatfield, PA). Excess solvent was removed by touching the edge of the grids with a small piece of filter paper (Whatman-1). The grids were allowed to dry at room temperature followed by staining with a drop of 2 wt% uranyl acetate (freshly prepared in Nano pure water and filtered through a 0.2 μm filter membrane) was added to the dry samples on the grids. After 2 min, excess staining agent was removed by filter paper, and the grids were further dried at ambient temperature for 15 min and used for TEM imaging.

DLS measurements were performed on a Zeta Potential and Particle Size Analyzer (ZetaPALS, Brookhaven Instruments Corp., US). Dust-free vials were used for the aqueous solutions. Measurements were made at 25^oC with a scattering angle of 90^o. For each sample, five replicates were obtained to determine mean sizes and size distribution. Number average diameters from cumulant analysis of correlation function assuming lognormal size distribution are reported.

Synthesis of 4-vinylbenzaldehyde. A solution of 4-vinylbenzyl chloride (10 g, 65 mmol) and hexamethylenetetramine (9.8 g, 70 mmol) in chloroform (100 mL) was stirred overnight at room temperature. Then water (200 mL) was added and the organics was distilled off at bath temperature ca. 130ºC until the distillate became clear. The distillate was acidified with 1N HCl to pH=5 and extracted with DCM (3×100 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using 5% Et_2O in hexanes as an eluent to give pure 4-vinylbenzaldehyde as colorless liquid 4.08g (47%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.40 (dd, J = 10.9, 0.5 Hz, 1H, CH=CH*H*), 5.87 (dd, J = 17.6, 0.5 Hz, 1H, CH=C*H*H), 6.73 (dd, J = 17.6, 10.9 Hz, 1H, C*H*=CH_2), 7.51 (d, J = 8.3 Hz, 2H, 2×CH_{ar}), 7.80 (d, J = 8.3 Hz, 2H, 2×CH_{ar}), 9.95 (s, 1H, CH=O); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 117.29 (CH=*C*H_2), 126.58 (2×CH_{ar}), 129.91 (2×CH_{ar}), 135.52 (C_{ar}), 135.72 (*C*H=CH_2), 143.26 (C_{ar}), 191.50 (CH=O).

Synthesis of DIBO-Pyrene derivative 15. A solution of 1-Pyrenemethylamine hydrochloride (100 mg, 0.37 mmol), carbonic acid 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl ester[15a] (144 mg, 0.37 mmol) and *N*,*N*-diisopropylethylamine (0.13 mL, 0.74 mmol) in DCM (5mL) was stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel using a gradient of 10 to 25% ethyl acetate in hexanes to give DIBO-Pyrene derivative **15** (145 mg, 82%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.90 (dd, J = 15.0, 3.9 Hz, 1H, CHCH*H*), 3.14 (dd, J = 15.0, 2.1 Hz, 1H, CHC*H*H), 5.09 (dq, J = 14.6, 5.4 Hz, 2H, C*H*$_2$NH), 5.35 (t, J = 5 Hz, 1H, NH), 5.60-5.61 (m, 1H, C*H*CH_2), 7.27-7.49 (m, 8H, 8×CH_{ar}), 7.95-8.29 (m, 9H, 9×CH_{ar}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 43.83 (CH_2NH), 46.44 (*C*H_2CH), 77.37 (*C*HCH_2), 110.23 (*C*≡C), 113.19 (C≡*C*), 121.55 (C_{ar}), 123.06 (CH_{ar}), 123.97

(CH_{ar}), 124.12 (C_{ar}), 125.03 (CH_{ar}), 125.31 (CH_{ar}), 125.61 (CH_{ar}), 125.69 (CH_{ar}), 126.24 (CH_{ar}), 126.36 (CH_{ar}), 126.50 (CH_{ar}), 127.25 (C_{ar}), 127.33 (2×CH_{ar}), 127.56 (C_{ar}), 127.59 (CH_{ar}), 127.83 (CH_{ar}), 128.14 (CH_{ar}), 128.30 (CH_{ar}), 128.52 (CH_{ar}), 129.22 (C_{ar}), 129.26 (C_{ar}), 130.17 (CH_{ar}), 130.97 (C_{ar}), 131.50 (C_{ar}), 131.52 (C_{ar}), 151.22 (C_{ar}), 152.22 (C=O), 155.41 (C_{ar}); **HRMS** (MALDI) 477.01 ($C_{34}H_{23}NO_2$ (M^+) requires 477.17).

General procedure for synthesis of PEG azides. A solution of respective poly(ethylene glycol) methyl ether (10 mmol), triethylamine (2.75 mL, 20 mmol), *p*-toluenesulfonyl chloride (2.85 g, 15 mmol) in DCM (100 mL) was stirred overnight at room temperature. The reaction mixture was diluted with 100 mL of DCM and washed with 1N HCl (100 mL), aq $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was dried overnight and added to a suspension of sodium azide (1.3 g, 20 mmol) in ethanol (150 mL). The reaction mixture was refluxed overnight, and then diluted with water (100 mL). Ethanol was removed from the mixture under the reduced pressure. The aqueous layer was extracted with DCM (3×100 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of 3 to 10 % MeOH in DCM as eluent to give pure PEG azides as white solids ($PEG_{750}N_3$ melts around room temperature).

$PEG_{750}N_3$ yield (5.85g, 78%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.36-3.39 (m, 5H, CH_3O, CH_2N_3), 3.51-3.55 (m, 2H, C*H*$_2$$CH_2N_3$), 3.61-3.68 (m, 60H, 30×CH_2O); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 50.64 (CH_2N_3), 58.98 (CH_3O), 69.98 (*C*$H_2CH_2N_3$), 70.52 (29×CH_2O), 71.88 (CH_2O); **HRMS** (MALDI) 740.25 ($C_{36}H_{74}NaN_6O_{15}$(MNa^+), n=14 requires 740.42).

$PEG_{2000}N_3$ yield (12.80g, 64%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.38-3.40 (m, 5H, CH_3O, CH_2N_3), 3.54-3.56 (m, 2H, C*H*$_2$$CH_2N_3$), 3.65-3.69 (m, 172H, 86×CH_2O); **^{13}C NMR** (75.5 MHz,

$CDCl_3$) δ 50.48 (CH_2N_3), 58.82 (CH_3O), 69.83 ($CH_2CH_2N_3$), 70.37 (85×CH_2O), 71.73 (CH_2O); **HRMS** (MALDI) 1884.79 ($C_{83}H_{167}NaN_3O_{41}$ (MNa^+), n=40 requires 1885.10).

General procedure for synthesis of PEG amines. A solution of a respective PEG azide (1 mmol) and triphenylphosphine (2 mmol) in THF:water mixture 1:1 (50 mL) was stirred overnight at room temperature. Then THF was removed under reduced pressure and the resulting precipitate was filtered off. The aqueous supernatant was basified by addition of 1N NaOH solution (50 mL) and extracted with DCM (3×100 mL). The organic extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of 10 to 15% methanol in DCM:NH_4OH 100:1 mixture to give pure PEG amines as white solids.

$PEG_{750}NH_2$ yield (0.65g, 86%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 1.97 (brs, 2H, CH_2NH_2), 2.84 (t, J = 4.6 Hz, 2H, CH_2NH_2), 3.35 (s, 3H, CH_3), 3.48-3.52 (m, 4H, 2×CH_2O), 3.61 (s, 58H, 29×CH_2O); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 41.67 (CH_2NH_2), 58.92 (CH_3O), 70.18 (CH_2O), 70.46 (28×CH_2O), 71.83 (CH_2O), 73.19 ($CH_2CH_2NH_2$); **HRMS** (MALDI) 758.33 ($C_{33}H_{69}NaNO_{16}$ (MNa^+), n=15 requires 758.45).

$PEG_{2000}NH_2$ yield (0.98g, 49%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.47 (brs, 2H, CH_2NH_2), 2.91 (t, J = 4.8 Hz, 2H, CH_2NH_2), 3.38 (s, 3H, CH_3), 3.54-3.58 (m, 4H, 2×CH_2O), 3.61 (s, 170H, 85×CH_2O); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 41.54 (CH_2NH_2), 58.96 (CH_3O), 70.17 (CH_2O), 70.49 (84×CH_2O), 71.86 (CH_2O), 72.42 ($CH_2CH_2NH_2$); **HRMS** (MALDI) 1859.36 ($C_{83}H_{169}NaNO_{41}$ (MNa^+), n=40 requires 1859.11).

General procedure for synthesis of DIBO-PEG derivatives 16, 17. A solution of a respective PEG amine (0.3 mmol) carbonic acid 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl ester **S1** (115 mg, 0.3 mmol), *N*,*N*-

diisopropylethylamine (0.16 mL, 0.9 mmol) in DCM (10mL) was stirred overnight at room temperature. Then the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel using a gradient of 0 to 10% methanol in DCM to give pure PEG-DIBO.

PEG_{750}-DIBO 16. Colorless oil (240 mg, 86%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.90 (d, *J* = 15.0 Hz, 1H, CHCH*H*), 3.18 (d, *J* = 15.0 Hz, 1H, CHC*H*H), 3.38-3.40 (m, 5H, C*H*$_2$NH, CH_3O), 3.55-3.69 (m, 62H, 31×CH_2O), 5.50 (brs, 1H, C*H*CH_2), 5.64 (brs, 1H, NH), 7.27-7.35 (m, 7H, 7×CH_{ar}), 7.52 (d, 1H, CH_{ar}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 40.96 (CH_2NH), 46.18 (*C*H_2CH), 59.03 (CH_3O), 70.02 (CH_2O), 70.33 (CH_2O), 70.57 (27×CH_2O), 71.93 (CH_2O), 76.77 (*C*HCH_2), 109.98 (*C*≡C), 112.89 (C≡*C*), 121.28 (C_{ar}), 123.78 (CH_{ar}), 123.83 (C_{ar}), 125.92 (CH_{ar}), 126.20 (C_{ar}), 127.01 (CH_{ar}), 127.03 (CH_{ar}), 127.91 (CH_{ar}), 128.03 (CH_{ar}), 129.92 (CH_{ar}), 151.03 (C_{ar}), 152.20 (C=O), 155.50 (C_{ar}); **HRMS** (MALDI) 1004.30 ($C_{50}H_{79}NaNO_{18}$ (MNa^+), n=15 requires 1004.52).

PEG_{2000}-DIBO 17. White solid (498 mg, 65%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 2.90 (dd, *J* = 15.0, 3.4 Hz, 1H, CHCH*H*), 3.18 (dd, *J* = 15.0, 1.8 Hz, 1H, CHC*H*H), 3.38-3.41 (m, 5H, C*H*$_2$NH, CH_3O), 3.54-3.69 (m, 174H, 87×CH_2O), 5.50 (brs, 1H, C*H*CH_2), 5.64 (brs, 1H, NH), 7.28-7.37 (m, 7H, 7×CH_{ar}), 7.52 (d, *J* = 7.6 Hz, 1H, CH_{ar}); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 40.91 (CH_2NH), 46.13 (*C*H_2CH), 58.97 (CH_3O), 70.00 (CH_2O), 70.25 (CH_2O), 70.50 (84×CH_2O), 71.87 (CH_2O), 76.72 (*C*HCH_2), 109.93 (*C*≡C), 112.83 (C≡*C*), 121.23 (C_{ar}), 123.74 (CH_{ar}), 123.78 (C_{ar}), 125.87 (CH_{ar}), 126.15 (C_{ar}), 126.96 (CH_{ar}), 126.98 (CH_{ar}), 127.87 (CH_{ar}), 127.98 (CH_{ar}), 129.88 (CH_{ar}), 150.98 (C_{ar}), 152.15 (C=O), 155.50 (C_{ar}); **HRMS** (MALDI) 2105.23 ($C_{100}H_{179}NaNO_{43}$ (MNa^+), n=40 requires 2105.17).

Synthesis of 2-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside S3. Silver trifluoromethanesulfonate (3.0 g, 11.7 mmol) was added to a stirred solution of α-D-lactosylbromide heptaacetate[34] **S2** (4.0 g, 5.73 mmol) and 5-azidopentanol[32] (1.5 g, 11.63 mmol) in dry DCM (30 mL) containing 3Å molecular sieves (5 g) under the atmosphere of argon at -78ºC. The reaction mixture was stirred for 2 h and then allowed to warm up to room temperature. The reaction was quenched by addition of Et_3N:MeOH mixture (5 mL, 1:1, v/v). The solution was then filtered through celite pad and concentrated. The residue was purified by column chromatography on silica gel using 50% ethyl acetate in hexanes as an eluent to give pure lactose azide **S3** (1.7 g, 40%) as colorless oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 1.36-1.44 (m, 2H, CH_2), 1.55-1.61 (m, 4H, 2×CH_2), 1.95 (s, 3H, CH_3), 2.03-2.05 (m, 12H, 4×CH_3), 2.11 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 3.25 (t, J = 6.8 Hz, 2H, CH_2N_3), 3.46 (td, J = 9.7, 6.6 Hz, 1H, OC*H*HCH_2), 3.59 (ddd, J = 9.8, 5.0, 1.9 Hz, 1H, C*H*CH_2), 3.76-3.88 (m, 3H, OCH*H*CH_2, C*H*CH_2, CHO), 4.05-4.14 (m, 3H, CHC*H*$_2$O, CHC*H*HO), 4.44-4.49 (m, 3H, 2×CHO, CHCH*H*O), 4.87 (dd, J = 9.5, 8.02 Hz, 1H, CHO), 4.94 (dd, J = 10.4, 3.44 Hz, 1H, CHO), 5.09 (dd, J = 10.3, 7.95 Hz, 1H, CHO), 5.18 (t, J = 9.3 Hz, 1H, CHO), 5.33 (d, J = 3.18 Hz, 1H, CHO); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 20.43 (CH_3), 20.55 (3×CH_3), 20.59 (CH_3), 20.73 (CH_3), 20.78 (CH_3), 23.06 (CH_2), 28.43 (CH_2), 28.86 (CH_2), 51.25 (CH_2), 60.73 (CH_2O), 61.93 (CH_2O), 66.55 (CHO), 69.05 (CHO), 69.60 (CH_2O), 70.60 (*C*HCH_2), 70.91 (CHO), 71.62 (CHO), 72.55 (CHO), 72.75 (*C*HCH_2), 76.22 (CHO), 100.47 (CHO), 100.99 (CHO), 168.99 (CO), 169.51 (CO), 169.71 (CO), 169.96 (CO), 170.06 (CO), 170.26 (CO), 170.27 (CO); **HRMS** (MALDI) 770.25 ($C_{31}H_{45}NaN_3O_{18}$ (MNa^+) requires 770.26).

Synthesis of 2-azidopentyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside S4. A solution of 2-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-

glucopyranoside **S3** (1g, 1.34 mmol) in MeOH (15 mL) was basified to pH=10 using catalytic amount of potassium tert-butoxide. The reaction mixture was stirred for 2 h at room temperature and then neutralized using Dowex 50WX8 ion exchange resin. The resin was filtered off and the solvent was concentrated. The resulting residue was purified by column chromatography on silica gel using 20% MeOH in DCM as an eluent to give pure 2-azidopentyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **S4** (387 mg, 64%) as an amorphous solid. **^{1}H NMR** (600 MHz, D_2O) δ 1.41-1.46 (m, 2H, CH_2), 1.60-1.68 (m, 4H, 2×CH_2), 3.28-3.34 (m, 3H, C*H*OH, CH_2N_3), 3.53 (dd, J = 9.9, 7.9 Hz, 1H, C*H*OH), 3.56-3.60 (m, 1H, C*H*CH_2OH), 3.61-3.73 (m, 5H, OCH*H*CH_2, C*H*OH, 2×C*H*OH, C*H*CH_2OH), 3.74-3.80 (m, 3H, C*H*$_2$OH, C*H*HOH), 3.90-3.94 (m, 2H, CHO, OC*H*HCH_2), 3.97 (dd, J = 12.2, 2.0 Hz, 1H, CH*H*OH), 4.44 (d, J = 7.8 Hz, 1H, CHO), 4.47 (d, J = 8.0 Hz, 1H, CHO); **^{13}C NMR** (75.5 MHz, D_2O) δ 21.20 (CH_2), 26.51 (CH_2), 27.08 (CH_2), 49.87 (CH_2), 58.90 (CH_2OH), 59.81 (CH_2OH), 67.34 (CHOH), 69.16 (CH_2O), 69.75 (CHOH), 71.32 (CHOH), 71.63 (CHOH), 73.24 (*C*HCH_2), 73.55 (*C*HCH_2), 74.15 (CHOH), 77.21 (CHO), 100.82 (CHO), 101.72 (CHO); **HRMS** (MALDI) 476.17 ($C_{17}H_{31}NaN_3O_{11}$ (MNa^+) requires 476.19).

Synthesis of DIBO-Lactose 18. A solution of 2-azidopentyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (200 mg, 0.44 mmol) **S4** and trimethylphosphine (4.4 mL as 1M solution in THF, 4.4 mmol) in THF/H_2O mixture (10 mL, 2:1, v/v) was stirred for 3 h at room temperature. The solvent was removed under reduced pressure and the residue containing 2-azidopentyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**HRMS** (MALDI) 450.23 ($C_{17}H_{33}NaNO_{11}$ (MNa^+) requires 450.19) was coevaporated with toluene (3×10 mL). After drying overnight under reduced pressure the flask containing the amine residue was charged with carbonic acid 11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*]cycloocten-5-yl 4-nitrophenyl ester **S1** (169 mg, 0.44 mmol),

N,*N*-diisopropylethylamine (0.15 mL, 0.88 mmol) and DMF (10 mL) and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel using a gradient of 15 to 25% MeOH in DCM to give pure DIBO-Lactose **18** (242 mg, 82%). **^{1}H NMR** (500 MHz, CD_3OD) δ 1.41-1.50 (m, 2H, CH_2), 1.53-1.59 (m, 2H, CH_2), 1.60-1.68 (m, 2H, CH_2), 2.84 (dd, J = 15.0, 3.9 Hz, 1H, CH*H*CHO), 3.13 (t, J = 6.85 Hz, 2H, CH_2NH), 3.21-3.27 (m, 2H, C*H*OH, C*H*HCHO), 3.39-3.41 (m, 1H, C*H*CH_2OH), 3.48-3.61 (m, 6H, 4×C*H*OH, C*H*CH_2OH, OCH*H*CH_2), 3.71 (dd, J = 11.4, 4.7 Hz, C*H*HOH), 3.77-3.93 (m, 5H, CHO, C*H*$_2$OH, OC*H*HCH_2, OC*H*HCH_2), 4.29 (d, J = 7.8 Hz, 1H, CHO), 4.37 (dd, J = 7.6, 1.0 Hz, 1H, CHO), 5.43 (brs, 1H, CH_2C*H*O), 7.30-7.43 (m, 7H, 7×CH_{ar}), 7.57 (d, J = 7.7, Hz, CH_{ar}); **^{13}C NMR** (75.5 MHz, CD_3OD) δ 24.33 (CH_2), 30.41 (CH_2), 30.63 (CH_2), 41.81 (CH_2), 47.25 (*C*H_2CHO), 61.99 (CH_2OH), 62.53 (CH_2OH), 70.34 (CHOH), 70.74 (CH_2O), 72.61 (CHOH), 74.82 (CHOH), 74.87 (CHOH), 76.48 (*C*HCH_2OH), 76.53 (*C*HCH_2OH), 77.13 (CHOH), 77.87 (CH_2C*H*O), 80.74 (CHO), 104.29 (CHO), 105.14 (CHO), 111.04 (C≡*C*), 113.86 (*C*≡C), 122.47 (C_{ar}), 124.98 (CH_{ar}), 125.02 (C_{ar}), 126.93 (CH_{ar}), 127.21 (C_{ar}), 128.29 (CH_{ar}), 128.35 (CH_{ar}), 129.29 (CH_{ar}), 129.39 (CH_{ar}), 151.03 (CH_{ar}), 152.51 (C_{ar}), 153.79 (C=O), 158.06 (C_{ar}); **HRMS** (MALDI) 696.28 ($C_{34}H_{43}NaNO_{13}$ (MNa^+) requires 696.26).

General random copolymerization procedure. A dry Shlenk flask was charged with styrene, 4-vinylbenzaldehyde or 4-vinylbenzyl chloride in a desirable proportion and 0.01 equivalents (relative to the monomer) of 2-cyano-2-propyl dodecyl trithiocarbonate or macro CTAs **7.** A stock solution containing 0.001 equivalents (relative to the monomer) of AIBN in dioxane was added to the mixture followed by 3 mL of dioxane. The flask was flushed with argon and the mixture was subjected to three freeze-pump-thaw cycles and then stirred for 20 h

at 70ºC. The polymerization was terminated by submersion into liquid nitrogen and exposure to air. Then the reaction mixture was diluted with THF (3 mL) and the polymer was purified by precipitation into cold methanol (250 mL) twice.

General dodecyltrithiocarbonate cleavage procedure. NMR-based M_n was used to calculate quantities of reagents for dodecyl trithiocarbonate cleavage. A dry Shlenk flask was charged with AIBN (20 equivalents relative to the polymer), BPO (2 equivalents relative to the polymer), a polymer with dodecyl trithiocarbonate chain end and 1,4-dioxane (5 mL). The flask was flushed with argon; the mixture was subjected to three freeze-pump-thaw cycles and stirred for 10 h at 80ºC. The reaction polymerization was terminated by submersion into liquid nitrogen and exposure to air. The reaction mixture was diluted with THF (3 mL) and the polymer was purified by precipitation into cold methanol (250 mL) twice.

General oximation procedure. A solution of aldehyde-containing polymer, hydroxylamine hydrochloride (2-3.5 equivalents per functional group) and triethylamine (4-7 equivalents per functional group) in DCM was stirred for 18 h at room temperature. The reaction mixture was diluted with DCM (50 mL), washed with water 3×10 mL, dried over $MgSO_4$ and concentrated. The polymer was purified by precipitation into cold hexanes.

General procedure for SPAAC reaction with DIBO derivatives. A solution of polymer and DIBO derivatives **15**-**18** (1.5-6 equivalents per azide group) in DCM was stirred for 18 h at room temperature. The polymer was purified by precipitation into appropriate solvent or treatment with azido-modified resin..

General procedure for SPANOC reaction with DIBO and DIBO derivatives 15, 16. A solution of (diacetoxyiodo)benzene (1.7-4 equivalents per oxime group) in DCM/MeOH mixture (5/1, v/v) was added dropwise to a stirred solution of oxime polymer and DIBO or

DIBO derivatives **15, 16** (2.5-6 equivalents per oxime group) in DCM/MeOH (5/1 v/v) and the resulting mixture was stirred for 4 h at room temperature. The solvent was evaporated under reduced pressure. The polymer was purified by precipitation into cold MeOH (for reactions with DIBO, **16**) or into cold Et_2O/Hexanes (1:2 v/v) mixture (for reactions with **15**).

Representative random copolymerization procedure. Synthesis of poly(4-vinylbenzaldehyde-*co*-styrene) 1b *via* RAFT polymerization. A dry Shlenk flask was charged with styrene (2.00 g, 19.20 mmol), VBA (127 mg, 0.96 mmol), AIBN (3.6 mg, 0.02 mmol), 2-cyano-2-propyl dodecyl trithiocarbonate (76 mg, 0.22 mmol) and 1,4-dioxane (3 mL). The mixture was subjected to three freeze-pump-thaw cycles and then stirred for 20 h at 70 ºC. The polymerization was terminated by submersion into liquid nitrogen. Then the reaction mixture was diluted with THF (3 mL) and the polymer was purified by precipitation in cold MeOH (250 mL) twice to give polymer **1b** (0.82g, 40%) as yellowish solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88 (t, J = 6.6 Hz, CH_3CH_2), 1.13-2.6 (m, CH_2CH_2, CHCH_2, CCH_3), 3.24 (brs, CH_2S), 4.67-4.95 (m, CHS), 6.37-7.62 (m, CH_{ar}), 9.89 (brs, CHO); M_n (g/mol) = 3400 (GPC), 4900 (NMR). PDI=1.09(GPC).

Representative dodecyltrithiocarbonate cleavage procedure. Synthesis of poly(4-vinylbenzaldehyde-*co*-styrene) 2b. A dry Shlenk flask was charged with AIBN (650 mg, 40 mmol), benzoyl peroxide (97 mg, 0.4 mmol), poly(4-vinylbenzaldehyde-*co*-styrene) polymer **1** (800 mg) and 1,4-dioxane (3 mL). The mixture was subjected to three freeze-pump-thaw cycles and then stirred for 10 h at 80ºC. The reaction mixture was diluted with THF (5 mL) and the polymer was purified by precipitation into cold MeOH (250 mL) twice to give a polymer **2b** (683 mg, 85%) as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.37 (m, CHCH_2, CCH_3), 6.37-7.52 (m, CH_{ar}), 9.89 (brs, CHO); M_n (g/mol) = 3250 (GPC, PDI=1.11).

Representative oximation procedure. Synthesis of poly(4-vinylbenzaldoxime-*co*-styrene) 3b. A solution of poly(4-vinylbenzaldehyde-*co*-styrene) **2b** (300 mg), hydroxylamine hydrochloride (52 mg, 0.75 mmol) and triethylamine (210 μL, 1.50 mmol) in DCM (5 mL) was stirred for 18 h at room temperature. The reaction mixture was diluted with DCM (50 mL), washed with water 3×10 mL, dried over $MgSO_4$ and concentrated. The polymer was purified by precipitation into cold hexanes 100 mL to yield **3** (230 mg, 77%) as white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.30 (m, C*H*C*H*$_2$, CCH_3), 6.36-7.34 (m, CH_{ar}), 8.08 (brs, C*H*NOH); M_n (g/mol) =3350 (GPC, PDI=1.10).

Representative procedure for preparation of azido-polymers. Synthesis of poly(1-(azidomethyl)-4-vinylbenzene-*co*-styrene) 9. A solution of sodium azide (117 mg, 1.8 mmol) and poly(1-(chloromethyl)-4-vinylbenzene-*co*-styrene) **8** (600 mg) in DMF (10 mL) was stirred overnight at room temperature. The resulting polymer was purified by precipitation into MeOH/water mixture (250 mL, 1/1, v/v) twice to give polymer **9** (482 mg, 80%) as white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.42 (m, C*H*C*H*$_2$, CCH_3), 4.21 (brs, CH_2N_3), 6.37-7.51 (m, CH_{ar}); M_n (g/mol) = 3350 (GPC, PDI=1.11).

Representative procedure for SPAAC reaction with DIBO. Synthesis of polymer 10. A solution of poly((1-azidomethyl)-4-vinylbenzene-*co*-styrene) **9** (40 mg) and DIBO (17 mg, 0.08 mmol) in DCM (5 mL) was stirred for 18 h at room temperature. The polymer was purified by precipitation into cold MeOH (50 mL) to yield polymer **10** as white solid (30 mg, 75%). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.86-2.15 (m, C*H*C*H*$_2$, CCH_3), 2.80-3.70 (m, C*H*$_2$CHO), 4.60-5.55 (m, CH_2N, CH_2CHO), 6.05-7.80 (m, CH_{ar}); M_n (g/mol) =3850 (GPC, PDI=1.10).

Representative procedure for SPANOC reaction with DIBO. Synthesis of polymer 4b. A solution of BAIB (19 mg, 0.06 mmol) in DCM/MeOH mixture (1.5 mL, 5/1 v/v) was

added dropwise to a stirred solution of polymer 3b (30 mg) and DIBO (20 mg, 0.09 mmol) in DCM/MeOH (5 mL, 5/1 v/v) the resulting mixture was stirred for 4 h at room temperature. The polymer was purified by precipitation into cold MeOH (50 mL) to yield polymer **4b** as white solid (22 mg, 73 %). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.93-2.15 (m, C*H*CH_2, CCH_3), 3.12-3.85 (m, CH_2CHO), 5.14-5.58 (m, CH_2C*H*O), 6.27-7.60 (m, CH_{ar}); M_n (g/mol) =3800 (GPC, PDI=1.10).

Poly(4-vinylbenzaldehyde-*co*-styrene) 1a. Prepared from styrene (5.00 g, 48.0 mmol), 4-vinylbenzaldehyde (209 mg, 1.58 mmol), AIBN (7.9 mg, 0.048 mmol), 2-cyano-2-propyl dodecyl trithiocarbonate (165 mg, 0.48 mmol) following general polymerization procedure. Polymer **1a** (2.02 g, 37%), yellowish solid. M_n (g/mol) = 3300 (GPC), 4800 (NMR). PDI=1.10 (GPC).

Poly(4-vinylbenzaldehyde-*co*-styrene) 1c. Prepared from styrene (2.00 g, 19.2 mmol), 4-vinylbenzaldehyde (507 mg, 3.84 mmol), AIBN (3.7 mg, 0.023 mmol), 2-cyano-2-propyl dodecyl trithiocarbonate (79.0 mg, 0.23 mmol) following general polymerization procedure. Polymer **1c** (1.26 g, 49%), yellowish solid. M_n (g/mol) = 4200 (GPC), 6300 (NMR). PDI=1.12 (GPC).

Poly(4-vinylbenzaldehyde-*co*-styrene) 2a. Prepared from polymer **1a** (280 mg), AIBN (200 mg, 1.2 mmol), BPO (30 mg, 0.12 mmol) following general dodecyltrithiocarbonate cleavage procedure. Polymer **2a** (225 mg, 80%), white solid. M_n (g/mol) = 3100 (GPC), PDI=1.09 (GPC).

Poly(4-vinylbenzaldehyde-*co*-styrene) 2c. Prepared from polymer **1c** (800 mg), AIBN (430 mg, 2.60 mmol), BPO (63 mg, 0.26 mmol) following general dodecyltrithiocarbonate

cleavage procedure. Polymer **2c** (706 mg, 88%), white solid. M_n (g/mol) = 3800 (GPC), PDI=1.14 (GPC).

Synthesis of poly(4-vinylbenzaldoxime-*co*-styrene) 3a. Prepared from polymer **2a** (225 mg), hydroxylamine hydrochloride (30 mg, 0.43 mmol) and triethylamine (120 μL, 0.86 mmol) following general oximation procedure. Polymer **3a** (153 mg, 68%), white solid. M_n (g/mol) =3250 (GPC, PDI=1.11).

Synthesis of poly(4-vinylbenzaldoxime-*co*-styrene) 3c. Prepared from polymer **2c** (300 mg), hydroxylamine hydrochloride (146 mg, 2.1 mmol) and triethylamine (580 μL, 4.2 mmol) following general oximation procedure. Polymer **3c** (212 mg, 71%), white solid. M_n (g/mol) =4550 (GPC, PDI=1.11).

Synthesis of polymer 4a. Prepared from (diacetoxyiodo)benzene (26 mg, 0.08 mmol), polymer **3a** (40 mg) and DIBO (26 mg, 0.12 mmol) following general procedure for SPANOC reaction with DIBO. Polymer **4a** (23 mg, 58%), white solid. M_n (g/mol) =3500 (GPC, PDI=1.11).

Synthesis of polymer 4c. Prepared from (diacetoxyiodo)benzene (33 mg, 0.10 mmol), polymer **3c** (15 mg) and DIBO (34 mg, 0.16 mmol) following general procedure for SPANOC reaction with DIBO. Polymer **4c** (13 mg, 56%), white solid. M_n (g/mol) =4900 (GPC, PDI=1.11).

Synthesis of nitrone bearing polymer 5. Prepared from polymer **2b** (200 mg), *N*-methyl hydroxylamine hydrochloride (50 mg, 0.6 mmol) and triethylamine (140 μL, 1.0 mmol) following general oximation procedure. Polymer **5** (160 mg, 80%), white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.30 (m, C*H*CH_2, CCH_3), 3.86 (br s, CH_3N), 6.36-7.34 (m, CH_{ar}), 7.90 (m, CH_{ar}); M_n (g/mol) =2800 (GPC, PDI=1.10).

Synthesis of polymer 6. Prepared from polymer **5** (40 mg) and DIBO (17 mg, 0.8 mmol) following a general procedure for SPAAC reaction. Polymer **6** (35 mg, 87%), white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.86-2.30 (m, C*H*CH_2, CCH_3), 2.80-3.00 (m, CH_2CHO, CH_3N), 4.80-5.40 (m, CH_2C*H*O, CHN), 6.05-7.80 (m, CH_{ar}); M_n (g/mol) =3500 (GPC, PDI=1.11).

Poly(4-vinylbenzyl chloride-*co*-styrene) 7. Prepared from styrene (7.00 g, 67.0 mmol), 4-vinylbenzyl chloride (1.02 g, 6.7 mmol), AIBN (12.0 mg, 0.073 mmol), 2-cyano-2-propyl dodecyl trithiocarbonate (252.0 mg, 0.73 mmol) following general polymerization procedure. Polymer **7** (3.47 g, 42%), yellowish solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88 (t, J = 6.6 Hz, C$H_3$$CH_2$), 1.13-2.6 (m, C$H_2CH_2$, C*H*C$H_2$, C$CH_3$), 3.24 (brs, CH_2S), 4.50 (br s, CH_2Cl), 4.67-4.95 (m, CHS), 6.37-7.30 (m, CH_{ar}); M_n (g/mol) = 3550 (GPC), 5500 (NMR). PDI=1.10 (GPC).

Poly(4-vinylbenzyl chloride-*co*-styrene) 8. Prepared from polymer **7** (800 mg), AIBN (420 mg, 2.6 mmol), BPO (65 mg, 0.26 mmol) following general dodecyltrithiocarbonate cleavage procedure. Polymer **8** (650 mg, 81%), white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 1.13-2.6 (m, CH_2CH_2, C*H*CH_2, CCH_3), 4.50 (br s, CH_2Cl), 6.37-7.30 (m, CH_{ar}); M_n (g/mol) = 3500 (GPC), PDI=1.12 (GPC).

Poly(4-vinylbenzyl chloride-*co*-styrene-*b*-4-vinylbenzaldehyde-*co*-styrene) 11. Prepared from styrene (5.00 g, 48.0 mmol), 4-vinylbenzaldehyde (0.32 g, 2.4 mmol), AIBN (8.2 mg, 0.05 mmol) and polymer **7** (2.70 g, 0.50 mmol (based on M_n=5500 g/mol (NMR)) following general polymerization procedure. Polymer **11** (4.49 g, 56%), yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, J = 6.6 Hz, C$H_3$$CH_2$), 1.13-2.6 (m, C$H_2CH_2$, C*H*C$H_2$, C$CH_3$), 3.24 (brs, CH_2S), 4.50 (br s, CH_2Cl), 4.67-4.95 (m, CHS), 6.37-7.62 (m, CH_{ar}), 9.89 (brs, CHO); M_n (g/mol) = 7700 (GPC), 11300 (NMR). PDI=1.15 (GPC).

Poly(4-vinylbenzyl chloride-*co*-styrene-*b*-4-vinylbenzaldehyde-*co*-styrene) 12. Prepared from polymer **11** (2.7 g), AIBN (0.98 g, 6.00 mmol), BPO (145 mg, 0.6 mmol) following general dodecyltrithiocarbonate cleavage procedure. Polymer **12** (2.59 g, 96%), white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 1.13-2.6 (m, CH_2CH_2, CHCH_2, CCH_3), 4.50 (br s, CH_2Cl), 6.37-7.62 (m, CH_{ar}), 9.89 (brs, CHO); M_n (g/mol) = 7300 (GPC), PDI=1.14 (GPC).

Synthesis of poly(1-(azidomethyl)-4-vinylbenzene-*co*-styrene-*b*-4-vinylbenzaldehyde-*co*-styrene) 13. A solution of sodium azide (0.62 g, 9.6 mmol) and poly(4-vinylbenzyl chloride-*co*-styrene) **8** (2.50 g) in DMF (15 mL) was stirred overnight at room temperature. The resulting polymer was purified by precipitation into methanol/water mixture (250 mL, 1/1, v/v) twice to give polymer **13** (2.24 g, 90%) as white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.42 (m, CHCH_2, CCH_3), 4.21 (brs, CH_2N_3), 6.37-7.51 (m, CH_{ar}), 9.89 (brs, CHO); M_n (g/mol) = 7600 (GPC, PDI=1.13).

Synthesis of poly(1-(azidomethyl)-4-vinylbenzene-*co*-styrene-*b*-4-vinylbenzaldoxime-*co*-styrene) 14. Prepared from polymer **13** (2.2 g), hydroxylamine hydrochloride (0.29 g, 4.23 mmol) and triethylamine (1.17 mL, 8.44 mmol) following general oximation procedure. Polymer **14** (2.08 g, 94 %), white solid.**^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.42 (m, CHCH_2, CCH_3), 4.21 (brs, CH_2N_3), 6.37-7.51 (m, CH_{ar}), 8.08 (brs, CHNOH); M_n (g/mol) =7100 (GPC, PDI=1.12).

Synthesis of polymer 19. Prepared from polymer **14** (320 mg) and DIBO-Pyrene **15** (300 mg, 0.63 mmol) following general procedure for SPAAC reaction with DIBO derivatives. Polymer **19** was purified by precipitating twice into cold Et_2O/hexanes mixture (150 mL, 1/2, v/v). Polymer **19** (402 mg, 95%), off white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 0.88-2.42 (m,

C*H*C*H*$_2$, CCH$_3$), 2.88-3.68 (m, C*H*$_2$CHO), 4.78-5.52 (m, CH$_2$N, CH$_2$C*H*O), 6.05-7.54 (m, CH$_{ar}$), 7.80-8.24 (m, CH$_{ar}$, C*H*NOH); M_n (g/mol) =7850 (GPC, PDI=1.13).

Synthesis of polymer 20. Prepared from (diacetoxyiodo)benzene (31 mg, 0.096 mmol), polymer **19** (50 mg) and DIBO-PEG$_{750}$ **16** (144 mg, 0.144 mmol) following general procedure for SPANOC reaction with DIBO derivatives. Polymer **20** (46 mg, 68%), off white solid. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 0.88-2.42 (m, C*H*C*H*$_2$, CCH$_3$), 2.70-4.00 (m, C*H*$_2$CHO, CH$_2$O, CH$_3$O), 4.60-5.80 (m, CH$_2$N, CH$_2$C*H*O, NH), 6.05-7.54 (m, CH$_{ar}$), 7.80-8.24 (m, CH$_{ar}$); M_n (g/mol) =10900 (GPC, PDI=1.23).

Synthesis of polymer 21a. Prepared from polymer **14** (100 mg) and DIBO-PEG$_{750}$ **16** (125 mg, 0.125 mmol) following general procedure for SPAAC reaction with DIBO derivatives. Polymer **21a** was purified by precipitating twice into cold MeOH. Polymer **21a** (130 mg, 85%), off white solid. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 0.88-2.42 (m, C*H*C*H*$_2$, CCH$_3$), 2.88-3.80 (m, C*H*$_2$CHO, CH$_3$O, CH$_2$O), 4.80-6.00 (m, CH$_2$N, CH$_2$C*H*O, NH), 6.05-7.60 (m, CH$_{ar}$), 8.10 (brs, C*H*NOH); M_n (g/mol) =9450 (GPC, PDI=1.14).

Synthesis of polymer 21b. Prepared from polymer **14** (50 mg) and DIBO-PEG$_{2000}$ **17** (83 mg, 0.036 mmol) following general procedure for SPAAC reaction with DIBO derivatives. The excess of DIBO-PEG reagent **17** was removed by treatment with azide resin in DCM overnight to give pure polymer **21b.** Polymer **21b** (62 mg, 74%), off white solid. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 0.70-2.48 (m, C*H*C*H*$_2$, CCH$_3$), 2.48-3.83 (m, C*H*$_2$CHO, CH$_3$O, CH$_2$O), 4.88-7.70 (m, CH$_2$N, CH$_2$C*H*O, NH), 5.93-7.65 (m, CH$_{ar}$), 8.03 (brs, C*H*NOH); M_n (g/mol) =12150 (GPC, PDI=1.12).

Synthesis of polymer 21c. Prepared from polymer **14** (100 mg) and DIBO-Lactose **18** (84 mg, 0.125 mmol) following general procedure for SPAAC reaction with DIBO derivatives.

Polymer **21a** (100 mg, 71%), off white solid. **^{1}H NMR** (500 MHz, DMF-*d6*) δ 0.71-2.34 (m, C*H*C*H*$_2$, CCH$_3$, CH$_2$), 2.82-3.82 (m, C*H*$_2$CHO, lactose C*H*OH, C*H*C*H*$_2$OH), 4.10-4.26 (m, lactose anomeric CHO), 4.46-4.54 (m, OH), 4.54-4.72 (m, OH), 4.99-5.18 (m, OH), 5.50-6.07 (m, CH$_2$N, CH$_2$C*H*O), 6.18-7.49 (m, CH$_{ar}$), 7.99 (brs, C*H*NOH); M_n (g/mol) =6850 (GPC, PDI=1.16).

Synthesis of polymer 22a. Prepared from (diacetoxyiodo)benzene (16 mg, 0.05 mmol), polymer **21a** (50 mg) and DIBO-Pyrene **15** (36 mg, 0.075 mmol) following general procedure for SPANOC reaction with DIBO derivatives. Polymer **22a** (20 mg, 50%), off white solid. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 0.83-2.13 (m, C*H*C*H*$_2$, CCH$_3$), 2.90-3.97 (m, C*H*$_2$CHO, CH$_2$O, CH$_3$O), 4.85-5.89 (m, CH$_2$N, CH$_2$C*H*O, NH), 6.11-7.70 (m, CH$_{ar}$), 7.85-8.36 (m, CH$_{ar}$); M_n (g/mol) =10550 (GPC, PDI=1.16).

Synthesis of polymer 22b. Prepared from (diacetoxyiodo)benzene (13 mg, 0.04 mmol), polymer **21b** (50 mg) and DIBO-Pyrene **15** (30 mg, 0.062 mmol) following general procedure for SPANOC reaction with DIBO derivatives. Polymer **22b** (46 mg, 77%), off white solid. **^{1}H NMR** (500 MHz, CDCl$_3$) δ 0.74-2.00 (m, C*H*C*H*$_2$, CCH$_3$), 2.83-3.75 (m, C*H*$_2$CHO, CH$_2$O, CH$_3$O), 4.75-5.92 (m, CH$_2$N, CH$_2$C*H*O, NH), 6.04-7.62 (m, CH$_{ar}$), 7.74-8.25 (m, CH$_{ar}$); M_n (g/mol) =15200 (GPC, PDI=1.16).

Synthesis of polymer 22c. Prepared from (diacetoxyiodo)benzene (16 mg, 0.05 mmol), polymer **21c** (50 mg) and DIBO-Pyrene **15** (36 mg, 0.075 mmol) following general procedure for SPANOC reaction with DIBO derivatives. Polymer **22c** (36 mg, 73%), off white solid. **^{1}H NMR** (500 MHz, DMF-*d6*) δ 0.84-2.48 (m, C*H*C*H*$_2$, CCH$_3$, CH$_2$), 2.94-3.99 (m, C*H*$_2$CHO, CH$_2$O, , lactose C*H*OH, C*H*C*H*$_2$OH, CH$_2$NH), 4.23-4.46 (m, lactose anomeric CHO), 4.60-4.70 (m, OH),

4.79-4.91 (m, OH), 5.00-5.90 (m, CH_2N, CH_2CHO, NH, OH, CH_2NH), 6.27-7.83 (m, CH_{ar}), 7.94-8.57 (m, CH_{ar}); M_n (g/mol) =7250 (GPC, PDI=1.18).

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CHAPTER 5

CONCLUSIONS

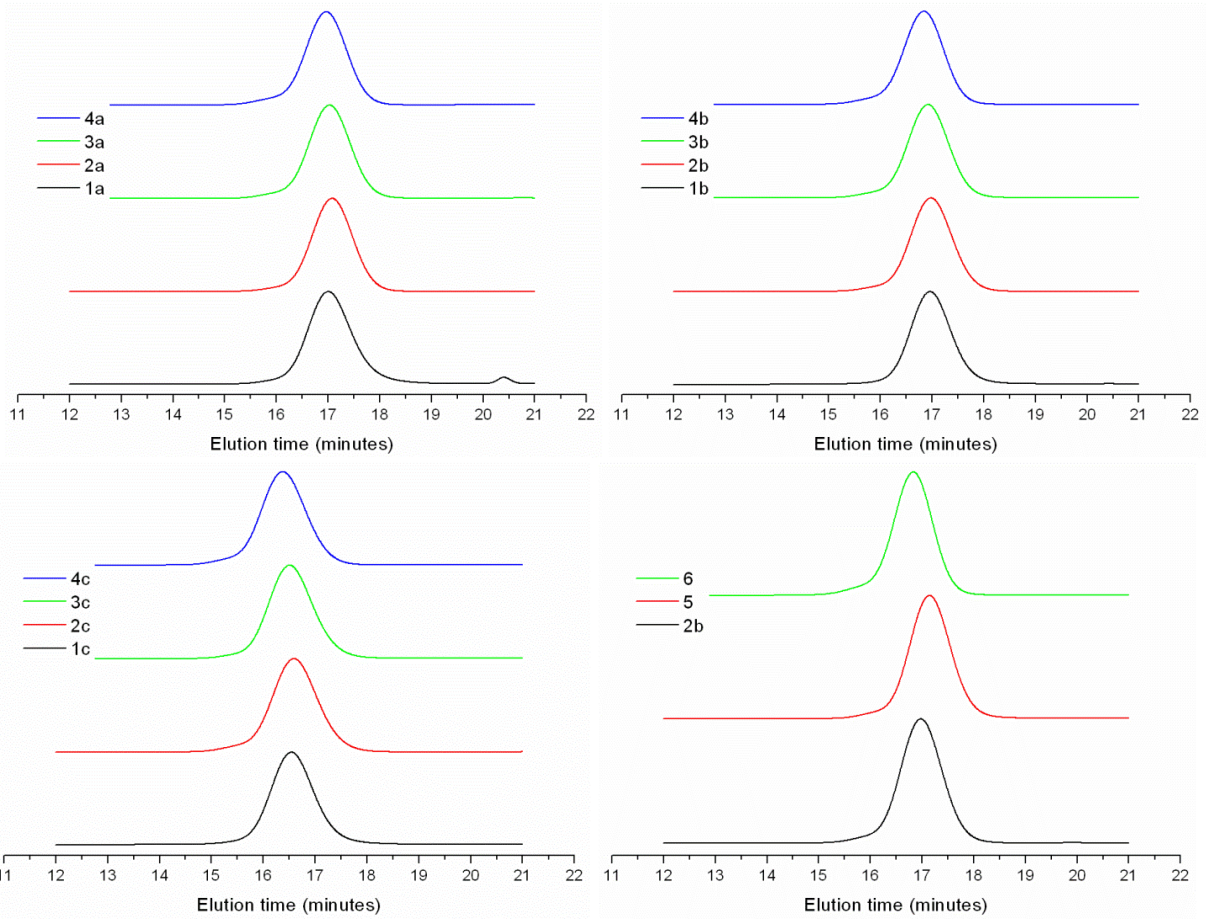
Dendritic scaffolds represent a valuable tool for studying carbohydrate-protein interactions due to the multivalent presentation of carbohydrates to lectins. Glycodendrimers can potentially be used as drug delivery vehicles and therefore a multi-functionalization to combine several moieties for targeting, imaging, and therapeutic effect in one macromolecule are often required. In Chapter 2 we demonstrated that a macromolecules could be bi-functionalized using traditional CuAAC followed by a one-pot two-step procedure, in which the CuF_2 reagent removed a trimethylsilyl protecting group of an alkyne and catalyzed a cycloaddition of the resulting terminal alkyne with an azide. The procedure could efficiently be employed for the preparation of several bi-functional model compounds containing unprotected carbohydrates. Chapter 3 described the development of a convenient approach for dendrimer assembly and peripheral functionalization using three consecutive azide-alkyne cycloadditions. Strain promoted azide-alkyne cycloaddition was established as an effective and chemoselective method for coupling of dendrons to give symmetrical and asymmetrical dendrimers bearing alkynes on the periphery. Differentiated terminal and TMS-protected peripheral alkynes were efficiently modified with various combinations of model PEG, galactosyl, and peptide-azides, bearing no protecting groups. The methodology is compatible with compounds that are rich in chemical functionalities such as peptides, carbohydrates, and fluorescent tags. Furthermore, only three consecutive steps are required for dendron coupling and installation of two different surface entities. Recently, photo-, thiol-ene, and strain-promoted alkyne-nitrone “click” reactions have been introduced,

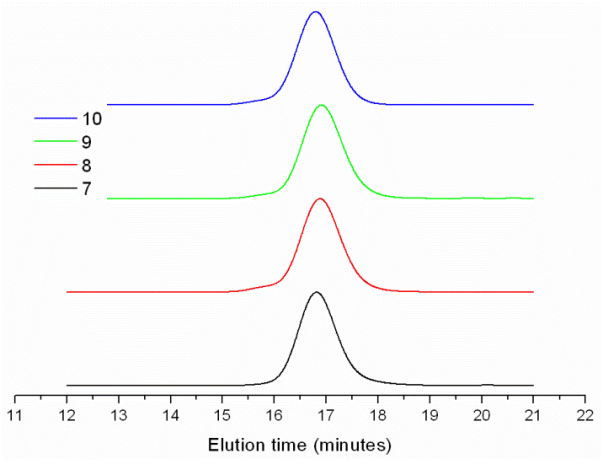
which also display excellent chemoselectivity and it is to be expected that integration of these reactions, in the approach reported here, will give easy access to even more complex dendritic structures. Strain promoted "click" reactions can be performed in a sequential manner by tuning the reactivity of 1,3-dipoles or by using a latent 1,3-dipole. We envisaged that sequential SPAAC/SPANOC reactions would facilitate the design and assembly of intricate polymeric structures for applications in material science and biomaterials. In Chapter 4, we demonstrated that block copolymers containing both azide and oxime moieties in segregated blocks could be prepared and derivatized with hydrophilic and hydrophobic moieties to give amphiphilic bottlebrush copolymers. The modular nature of this synthetic approach makes it possible to determine in a facile manner the optimal hydrophilic-hydrophobic balance for self-assembly of the resulting polymers in aqueous medium. This is the first time the SPANOC and SPANC were employed for post-polymerization modification of polymers. Future studies to extend this chemistry to thin films are underway. We expect that the search for orthogonal "click" reactions for multi-functionalization of materials will continue. The orthogonal [4+2] inverse electron demand Diels–Alder reaction between a trans-cyclooctene and a tetrazine and strain-promoted [3+2] cycloadditions between cyclooctynes and 1,3-dipoles may become a candidate for development of such sequential modifications.

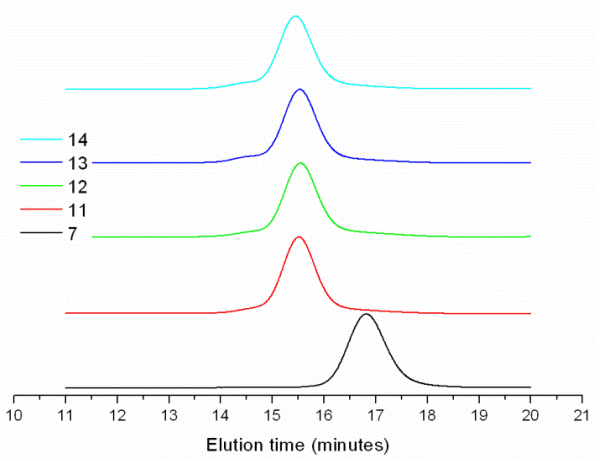
APPENDICES

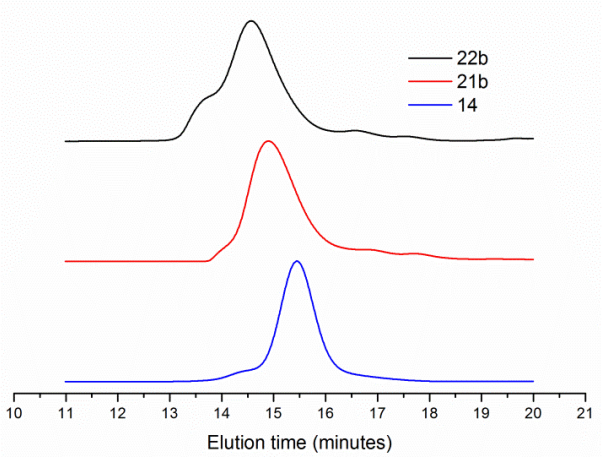
SUPPLEMENTARY INFORMATION FOR CHAPTER 4

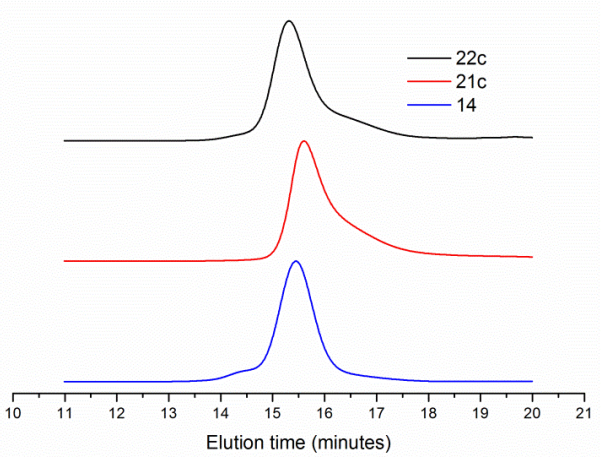
GPC traces of polymers



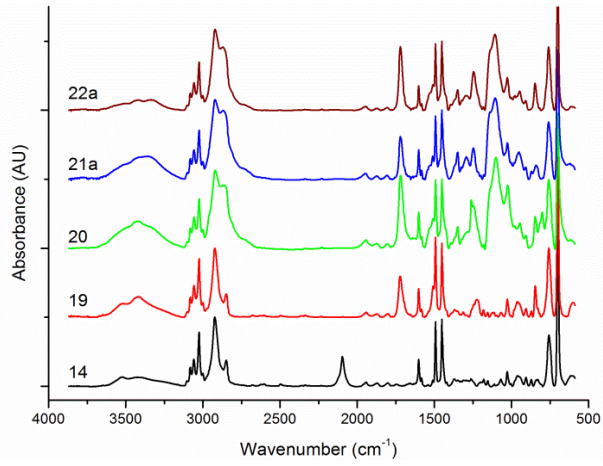


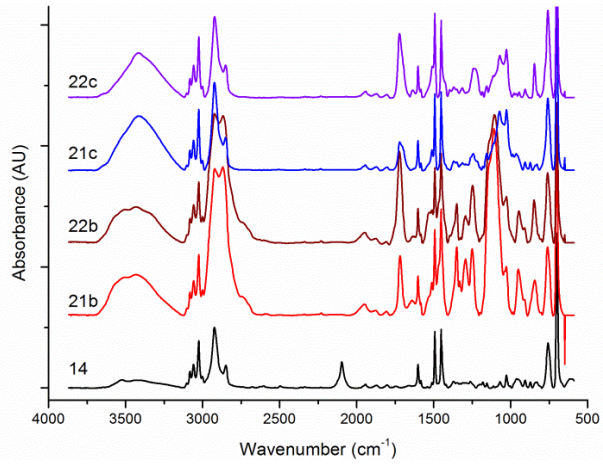






IR spectra of polymers





METAL-FREE SEQUENTIAL [3+2] CYCLOADDITIONS USING CYCLOOCTYNES AND 1,3-DIPOLES OF DIFFERENT REACTIVITY[†]

[†]Adapted with permission from: Sanders, B. C.; Friscourt, F.; Ledin, P. A.; Mbua, N. E.; Arumugam, S.; Guo, J.; Boltje, T. J.; Popik, V. V.; Boons, G. J. *J. Am. Chem. Soc.* **2011**, *133*, 949-957.

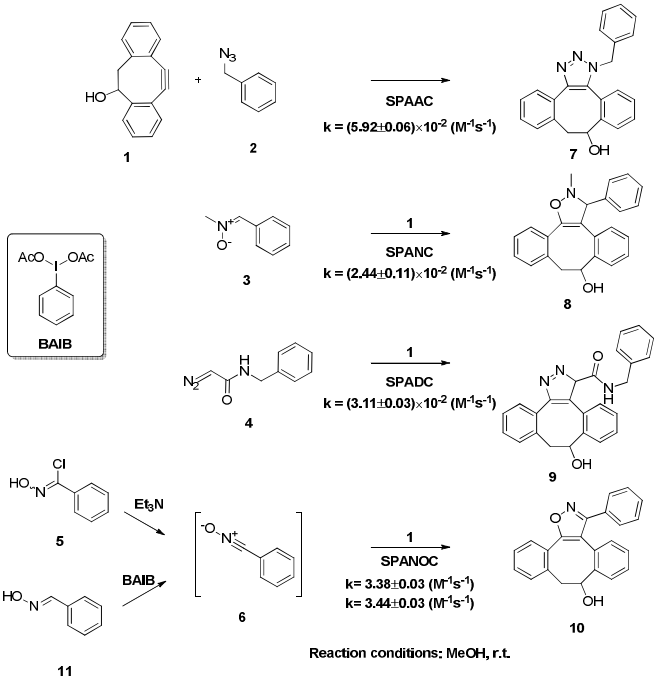
Introduction

Strained cyclooctynes react with azides under ambient conditions, without need for catalysis, *via* strain-promoted alkyne-azide cycloaddition (SPAAC).[1] In particular, the derivatives of 4-dibenzocyclooctynol (DIBO) (**1**) (Scheme 1) react fast with azido-containing saccharides and amino acids and can be employed for visualizing metabolically labeled glycans of living cells.[2] Furthermore, the finding that cyclooctynes can undergo fast cycloadditions with nitrones has further expanded the scope of metal-free "click" reactions,[3] and the usefulness of this approach has been demonstrated by site-specific protein modification an *N*-terminal serine, which could easily be converted into a nitrone and then reacted with probe-modified dibenzocyclooctynes. More reactive 1,3-dipoles, such as nitrile oxides, react slowly with terminal alkynes to give 3,5-isoxazoles under ambient conditions.[5] The reaction can be accelerated by the use of activated dipolarophiles such as benzyne and norbornenes or by employing a Cu(I) catalyst.[4] Sanders *et al.* found that in addition to azides and nitrones, nitrile oxides and diazocarbonyl derivatives readily undergo cycloadditions with dibenzocyclooctyne to give stable isoxazoles and pyrazoles, respectively.[6] Imidoyl chlorides, which can be converted into nitrile oxides by treatment with a mild base, can be prepared by reactions of the

corresponding aldehydes with hydroxylamine,[7] followed by chlorination of the resulting oximes with *N*-chlorosuccinimide.[8] Thus, the formation of isoxazoles is achieved by addition of the imidoyl chlorides to a solution of DIBO in the presence of triethylamine. The rates of strain-promoted alkyne-nitrile oxide cycloadditions (SPANOC) and alkyne-diazocarbonyl (SPADC) with DIBO compare favourably with the rates of similar cycloadditions with azides (SPAAC) and nitrones (SPANC).[6] For example, the reaction rates of 1,3-dipolar cycloadditions of DIBO (**1**) with benzyl azide (**2**), nitrone **3,** diazocarbonyl derivative **4** and a nitrile oxide **6** derived from imidoyl chloride **5**, to give triazole **7**, *N*-methyl isoxazole **8**, pyrazole **9** and isoxazole **10**, respectively, are provided in Scheme 1. The azide, nitrone, and diazocarbonyl derivatives exhibit similar rates of strain-promoted 1,3-dipolar cycloaddition. However, the rate of cycloaddition of the nitrile oxide was fifty seven times faster than a similar reaction with benzyl azide. The nitrile oxide formation can be streamlined by a direct oxidation of oximes to nitrile oxides by using a mild oxidant such as (diacetoxyiodo)benzene (BAIB).[9] Measurements of rate constants for the tandem sequence of oxidation of oximes to nitrile oxides followed by 1,3-dipolar cycloaddition with **1** establishing that the cycloaddition is the rate-limiting step and highlighting that oxidation with BAIB is exceptionally fast.[6] For example, when benzaldehyde oxime **11** was employed, the rate constant of the reaction was 3.44 $M^{-1}s^{-1}$, which is almost the same to the value obtained when benzaldehyde imidoyl chloride was employed (3.38 $M^{-1}s^{-1}$). Because carbohydrates are involved in a wide variety of biological processes,[10] the multivalent saccharide derivatives modified with fluorescent probes or biotin for immobilization are important tools to study the intriguing properties of this class of biomolecules.[11] SPANOC reaction proved to be useful for tagging reducing end of oligosaccharides by a sequential reaction of an aldose form with

hydroxylamine to give an oxime, which could then be functionalized by reaction with DIBO derivatives in the presence of BAIB.

Scheme 1 Rate constants of cycloadditions of DIBO (**1**) with various 1,3-dipoles: nitrile oxide, azide, nitrone and diazocarbonyl derivatives



It is interesting to note that the use of BAIB did not oxidize primary hydroxyls of lactose or sulfur of biotin, confirming a high chemoselectivity of SPANOC reaction.[12] In addition, a SPANOC reaction can also be used for the installation of tags into sialic acid containing glycoproteins by mild treatment with $NaIO_4$ to form a C-7 aldehyde, which upon treatment with hydroxylamine will give an oxime that can be oxidized to a nitrile oxide for reaction with derivatives of DIBO. The advantage of such strategy over oxime or hydrazone formation is that tags can be installed into glycoproteins by stable isoxazoles linkages.[13]

Sequential "click" reactions have been reported by Cu(I)-catalyzed alkyne azide cycloaddition[3a, 14] (CuAAC) using terminal- and silyl-protected alkynes [15] and by exploiting the

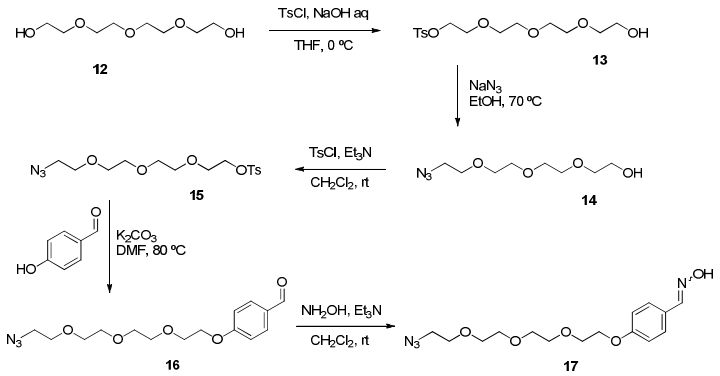
orthogonality of CuAAC with SPAAC and thiol-ene "click" reactions.[16] The usefulness of these approaches has been demonstrated by the controlled modification of oligonucleotides,[17] proteins[18] and fullerenes[19] with two or more tags. As has been stated previously various 1,3-dipoles exhibit distinct levels of reactivity making it possible to perform sequential cycloadditions. Furthermore, an oxime can function as a latent 1,3-dipole for a nitrile oxide, which is orthogonal with cycloadditions of azides. We, therefore, investigated the use of sequential strain-promoted cycloadditions for the assembly of complex multi-functional and bio-inspired materials without the need of employing a toxic metal catalyst.

Results and Discussion

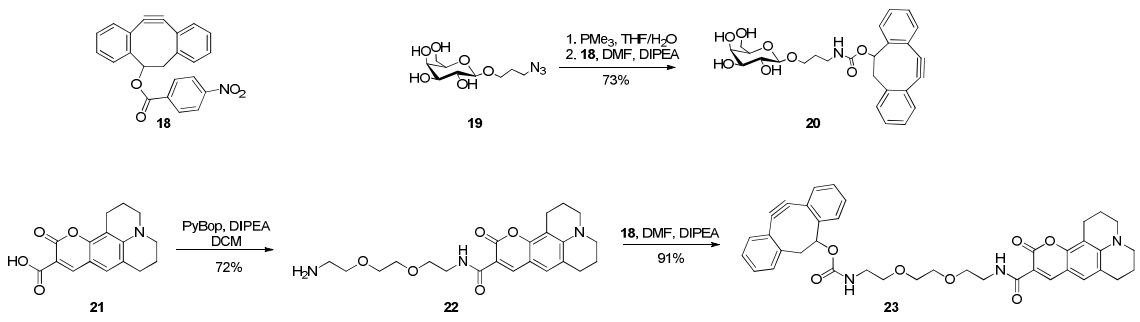
The large difference in reactivity of the cycloaddition of DIBO with the various 1,3-dipoles should make it possible to perform sequential "click" reactions, which may provide opportunities to prepare multi-functional compounds or materials by a simple synthetic procedure. In particular, it was expected that a highly reactive nitrile oxide can selectively undergo a cycloaddition in the presence of an azide. Furthermore, we envisaged that oximes can function as latent 1,3-dipoles, and therefore, a cyclooctyne should react with an azide without affecting an oxime. However, in the presence of BAIB, an oxime is rapidly converted into a nitrile oxide, which can then be reacted with another functionalized cyclooctyne. Thus, by careful selection of appropriate reagents, it should be possible to selectively modify a bi-functional linker (or complex compound) containing an azide and oxime moiety. In order to test this hypothesis, we have prepared a bi-functional oligoethyleneglycol-based derivative **17** having both azide and oxime groups (Scheme 2). The synthesis started from commercially available tetraethylene glycol **12,** which upon treatment with *p*-toluenesulfonylchloride gave monotosylated derivative **13.** Azido-group was installed by treatment of **13** with sodium azide to

give **14** in 81% yield. Aldehyde **16** was obtained by condensation of 4-hydroxybenzaldehyde with tosylate **15** in 60% yield in presence of potassium carbonate. Finally, oximation of **16** with hydroxylamine gave a bi-functional linker **17** in 88% yield. Both azides and nitrile oxides can undergo [3+2] cycloadditions with strained cyclooctyne derivatives. We, therefore, prepared DIBO-galactose derivative **20** from 3-azidopropyl β-D-galactopyranoside[20] by reducing the azide with trimethylphosphine and coupling the resulting amine to an 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester (**18**) (Scheme 3).[2] As expected, the addition of monosaccharide-modified DIBO **20** to bi-functional azido-oxime linker **17** in methanol resulted in selective cycloaddition at the azide moiety to provide the triazole **24** in high yield (Scheme 4). However, when linker **17** was treated with DIBO derivative **20** in the presence of BAIB, the oxime moiety was rapidly oxidized to a highly reactive nitrile oxide, which underwent a fast SPANOC resulting in the selective formation of isoxazole **25**. Having established the orthogonality of azides and oximes/nitrile oxides, we examined sequential SPAAC-SPANOC "click" reactions of bi-functional linker **17** with more complex glycoconjugates.

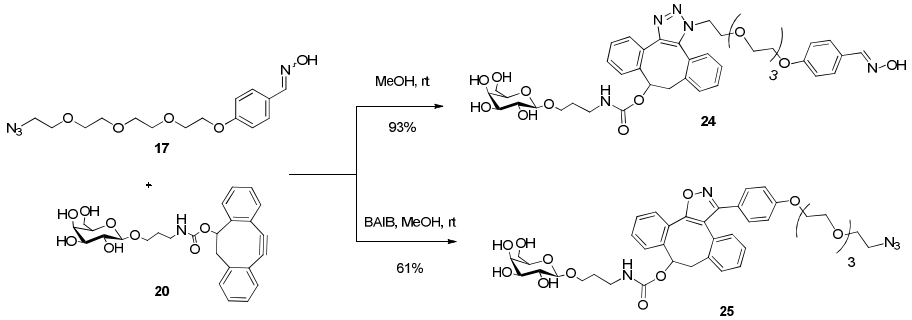
Scheme 2 Synthesis of a bi-functional linker **17**



Scheme 3 Synthesis of DIBO-derivatives **20** and **23**



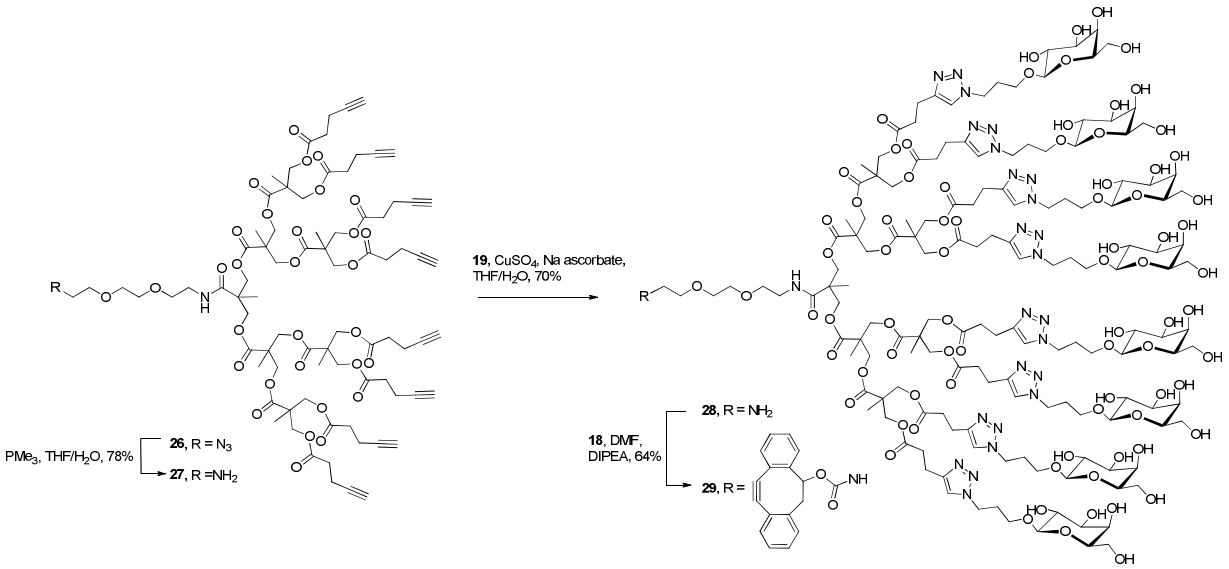
Scheme 4 Selective cycloadditions between galactoside-modified DIBO **20** with either the azide or oxime moiety of linker **17**



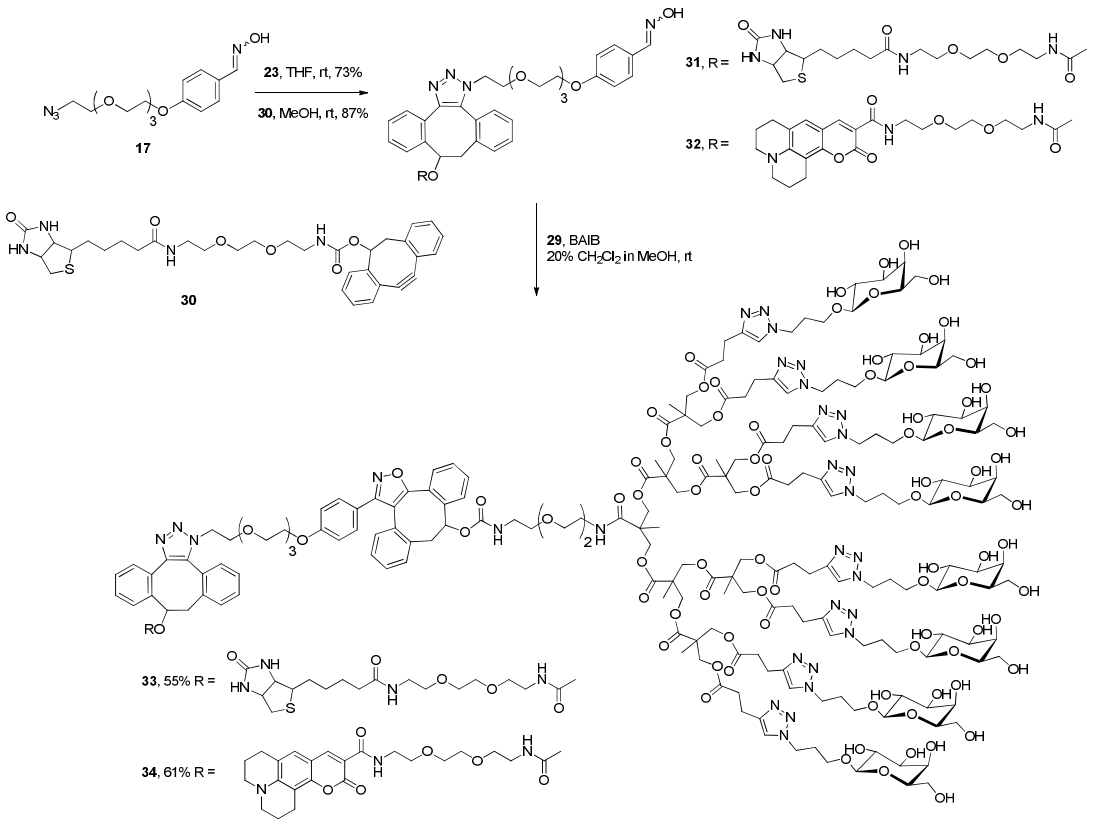
First, we prepared a DIBO-Coumarin 343 derivative by PyBop-mediated coupling of 2,2'-(ethylenedioxy)bis-(ethylamine) and commercially available acid coumarin derivative, followed by conversion of a primary amine of **22** into DIBO-carbamate **23** with excellent yields (Scheme 3). We have previously reported a convenient synthetic approach towards bi-functional polyester glycodendrimers based on bis-MPA.[15b] We used a third generation dendron **26** as a starting material for preparation of a galactose-bearing glycodendrimer with strained cyclooctyne at the focal point for subsequent conjugation to molecules containing 1,3-dipoles (Scheme 5). Since the modification of dendrimer periphery *via* CuAAC when the azide is present at the focal point is not possible, the glycosylation was performed after the reduction of the azide of **26** with

trimethylphosphine using an established procedure to give primary amine **27**. Cu(I)-mediated [3+2] cycloaddition between peripheral alkynes of **27** and unprotected galactoside **19** afforded glycodendrimer **28**. The free amine could be then used to selectively form a carbamate with 4-dibenzocyclooctynol upon treatment with **18** to give a reactive glycodendrimer **29** in 64% yield. Having a small library of reactive DIBO-derivatives in hand, we attempted a sequential hetero-bi-functionalization of a linker **17.** Thus, treatment of azido-oxime linker **17** with DIBO modified-biotin **30**[2] or DIBO modified–coumarin **23** in methanol or THF, respectively, at ambient temperature for 2 h, led to clean formation of mono-functionalized triazoles **31** and **32**, respectively (Scheme 6).

Scheme 5 Synthesis of glycodendron **29**



Scheme 6 Preparation of a bi-functional compound by a sequential SPAAC and SPANOC



Next, triazoles **31** and **32** were exposed to a mixture of BAIB to convert the oxime moiety into a highly reactive nitrile oxide, and reaction with DIBO-modified saccharide cluster **29**, lead to a fast SPANOC to give bi-functional compounds **33** and **34**, displaying a cluster of galactoses conjugated to biotin or a fluorescent tag, respectively. It is of interest to note that neither oxidation of biotin moiety by BAIB nor cycloaddition of the *in-situ* generated nitrile oxide at the carbon double bond of coumarin,[21] were observed, highlighting that SPANOC is perfectly suitable for the conjugation of sensitive compounds.

Conclusion

Strain-promoted cycloadditions between cyclooctynes and azides have been recognised as a powerful tool in chemical biology and material sciences. However, the search for faster and

more versatile bioorthogonal reactions continues. We have found that oximes and azides provide an orthogonal pair of functional groups for sequential metal-free "click" reactions. The results reported here demonstrate, that strain-promoted [3+2] cycloaddition reactions can be performed in a sequential manner by tuning the reactivity of 1,3-dipoles or by using a latent 1,3-dipole. The attractiveness of the new approach is that it offers chemical flexibility, avoids toxic metal catalysts, and makes it possible to multi-functionalize compounds by simple chemical manipulations. Metal-free "click" reactions have found entry into materials science, and it is to be expected that SPANOC will provide an additional tool for the preparation of increasingly complex materials by simple and flexible chemical manipulations. Finally, we anticipate that SPANOC will offer an attractive alternative to the well-established oxime ligation with biomolecules and a combined use with SPAAC will make it possible to introduce two different tags.

Experimental Section

All solvents were of reagent grade. All reagents were purchased from Sigma-Aldrich®. Room temperature refers to ambient room temperature (20-22 °C). Reactions were monitored by Thin Layer Chromatography (TLC) using aluminum backed silica gel 60 (F254) plates, visualized using UV254nm and potassium permanganate and ninhydrin dips as appropriate. Flash chromatography was carried out routinely using silica gel G60 (SiliCycle, 60-200μm 60 Å) as the stationary phase unless otherwise stated. The NMR spectra were recorded on a Varian Mercury (300 MHz) spectrometer. Due to the inseparable complex mixture of regioisomers and diastereoisomers of all "click" products, the ^{1}H-NMR and ^{13}C-NMR spectra were difficult to analyze in details. Therefore, only ^{1}H-NMR as well as HRMS are depicted. Chemical shifts are reported in δ units, parts per million (ppm) downfield from TMS. Coupling constants (J) are

measured in Hertz (Hz) and are unadjusted. Splitting patterns are designed as follows: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets, td – triplet of doublets, ddd – doublet of doublet of doublets, tt – triplet of triplets, sp – septet, m – multiplet, br – broad. Various 2D techniques were used to establish the structures and to assign the signals. High-resolution mass spectra were obtained by using either MALDI-ToF (Applied Biosystems 4700 Proteomics Analyzer) with 2,5-dihydroxybenzoic acid as a matrix. Reverse Phase HPLC purification was performed on an Agilent 1200 series system equipped with an automated injector, UV-detector, fraction-collector and Agilent Zorbax Eclipse XD8-C18 column (5 μm, 9.4 × 250 mm). The eluents used for all purifications were: A 0.1% TFA in water; B 0.1% TFA in CH_3CN, the flow was set to 1.5 ml/min.

2-(2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy)ethyl 4-methylbenzenesulfonate (13). Sodium hydroxide (0.69 g, 17.13 mmol, 1.6 equiv) was added to a solution of tetraethylene glycol (**12**) (21.95 g, 113 mmol, 10.3 equiv) in THF (5 mL) at 0 °C, followed by a slow addition of a solution of *p*-toluenesulfonylchloride (2.08 g, 10.93 mmol, 1.0 equiv) in THF (20 mL). The reaction mixture was then stirred for 2 h at 0 °C and poured into a mixture of ice and water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with water (2×50 mL), dried over $MgSO_4$ and evaporated *in vacuo* to yield **29** as a yellow oil (3.29 g, 86%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.33 (s, 3H, CH_3), 2.89 (t, *J* = 6.0 Hz, 1H, OH), 3.40-3.70 (m, 14H, 7×CH_2), 4.00-4.10 (m, 2H, C*H*$_2$OTs), 7.24 (d, *J* = 8.0 Hz, 2H, 2×Hm), 7.68 (d, *J* = 8.0 Hz, 2H, 2×Ho); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 21.41 (CH_3), 61.40 (*C*H_2OH), 68.44 (*C*H_2OTs), 69.17 (CH_2), 70.10 (CH_2), 70.22 (CH_2), 70.41 (CH_2), 70.46 (CH_2), 72.34 (CH_2), 127.73 (2×CHo), 129.68 (2×CHm), 132.76 (C), 144.68 (C).

2-(2-[2-(2-Azidoethoxy)ethoxy]ethoxy)ethanol (14). Sodium azide (3.0 g, 46.15 mmol, 5.0 equiv) was added to a solution of **13** (3.2 g, 9.18 mmol, 1.0 equiv) in ethanol (50 mL) at room temperature. The reaction mixture was stirred overnight at 70 °C. The reaction was then quenched by addition of water (50 mL), and concentrated under vacuum to a third of its volume. The aqueous layer was extracted with ethyl acetate (3×50 mL). The combined organic layers were then dried over $MgSO_4$ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of acetone and hexane (2:3) to yield **14** as an oil (1.63 g, 81%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.91 (t, *J* = 6.0 Hz, 1H, OH), 3.30 (t, *J* = 5.0 Hz, 2H, C*H*$_2$N$_3$), 3.49-3.65 (m, 14H, 7×CH_2); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 50.54 (*C*H_2N$_3$), 61.51 (*C*H_2OH), 69.91 (CH_2), 70.21 (CH_2), 70.46 (CH_2), 70.52 (CH_2), 70.56 (CH_2), 72.43 (CH_2).

2-(2-[2-(2-Azidoethoxy)ethoxy]ethoxy)ethyl 4-methylbenzenesulfonate (15). *p*-Toluenesulfonylchloride (2.13 g, 11.15 mmol, 1.5 equiv) was added to a solution of azide **14** (1.63 g, 7.43 mmol, 1.0 equiv) and triethylamine (1.55 mL, 11.15 mmol, 1.5 equiv) in CH_2Cl_2 (20 mL) at room temperature. The reaction mixture was stirred at room temperature for 6 h. Water (20 mL) was then added to the reaction mixture, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of 2% methanol in CH_2Cl_2 to afford pure **15** as an oil (2.47 g, 89%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.44 (s, 3H, CH_3), 3.38 (t, *J* = 5.0 Hz, 2H, C*H*$_2$N$_3$), 3.55-3.75 (m, 12H, 6×CH_2), 4.10-4.20 (m, 2H, C*H*$_2$OTs), 7.34 (d, *J* = 8.4 Hz, 2H, 2×Hm), 7.80 (d, *J* = 8.4 Hz, 2H, 2×Ho); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 21.77 (CH_3), 50.82

(CH_2N_3), 68.82 (CH_2), 69.37 (CH_2OTs), 70.18 (2×CH_2), 70.74 (CH_2), 70.81 (CH_2), 70.90 (CH_2), 128.11 (2×CHo), 129.94 (2×CHm), 133.16 (C), 144.92 (C).

4-(2-(2-[2-(2-Azidoethoxy)ethoxy]ethoxy)ethoxy)benzaldehyde (16). Potassium carbonate (1.36 g, 9.84 mmol, 1.5 equiv) was added to a solution of 4-hydroxybenzaldehyde (1.20 g, 9.84 mmol, 1.5 equiv) and azido-tosyl **15** (2.45 g, 6.56 mmol, 1.0 equiv) in DMF (10 mL) at room temperature. The reaction mixture was then stirred at 80 °C overnight. Water (15 mL) was then added to the reaction mixture, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The residue was then purified by flash column chromatography on silica gel using a mixture of of 2% methanol in CH_2Cl_2 to afford pure **16** as an oil (1.27 g, 60%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.37 (t, *J* = 5.0 Hz, 2H, CH_2N_3), 3.60-3.75 (m, 10H, 5×CH_2), 3.88 (t, *J* = 5.0 Hz, 2H, CH_2CH_2OAr), 4.21 (t, *J* = 5.0 Hz, CH_2CH_2OAr), 7.01 (d, *J* = 8.7 Hz, 2H, 2×Ho), 7.82 (d, *J* = 8.7 Hz, 2H, 2×Hm), 9.87 (s, 1H, CH=O); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 50.80 (CH_2N_3), 67.89 (CH_2OAr), 69.59 (CH_2 CH_2OAr), 70.17 (CH_2), 70.80 (CH_2), 70.84 (2×CH_2), 71.03 (CH_2), 115.00 (2×CHo), 130.17 (C), 132.08 (2×CHm), 163.98 (C), 190.95 (C=O).

4-(2-(2-[2-(2-Azidoethoxy)ethoxy]ethoxy)ethoxy)benzaldehyde oxime (17). *N*-Hydroxylamine hydrochloride (410 mg, 5.91 mmol, 1.5 equiv) was added to a solution of aldehyde **16** (1.27 g, 3.94 mmol, 1.0 equiv) and triethylamine (0.83 mL, 5.91 mmol, 1.5 equiv) in CH_2Cl_2 (10 mL) at room temperature. The reaction mixture was then stirred at room temperature overnight. Water (10 mL) was then added to the reaction mixture, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The residue was then purified by

flash column chromatography on silica gel using a mixture of 2% methanol in CH_2Cl_2 to afford pure **17** as an oil (1.17 g, 88%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.36 (t, *J* = 5.2 Hz, 2H, C*H*$_2$N$_3$), 3.63-3.76 (m, 10H, 5×CH_2), 3.86 (t, *J* = 5.0 Hz, 2H, C*H*$_2$CH$_2$OAr), 4.14 (t, *J* = 5.0 Hz, CH$_2$C*H*$_2$OAr), 6.89 (d, *J* = 8.7 Hz, 2H, 2×Ho), 7.46 (d, *J* = 8.7 Hz, 2H, 2×Hm), 8.06 (s, 1H, CH=N); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 50.79 (*C*H$_2$N$_3$), 67.60 (*C*H$_2$OAr), 69.75 (*C*H$_2$ CH$_2$OAr), 70.15 (CH_2), 70.79 (CH_2), 70.80 (CH_2), 70.82 (CH_2), 70.97 (CH_2), 114.99 (2×CHo), 124.96 (C), 128.55 (2×CHm), 149.86 (C=NOH), 160.35 (C).

DIBO-Galactose 20. 3-azidopropyl β-D-galactopyranoside[20] (93 mg, 0.35 mmol, 1 equiv) was dissolved in a mixture of THF and H_2O (9:1, v/v, 5 mL). A 1 M solution of trimethylphosphine in THF (1.75 mL, 1.75 mmol, 5 equiv) was added to the solution and the resulting mixture was stirred for 3 h. The solution was concentrated *in vacuo* and coevaporated with toluene (3×10 mL). The resulting residue was treated with 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester [2] (135 mg, 0.35 mmol, 1 equiv) and DIPEA (61 μL, 0.35 mmol, 1 equiv) in DMF (5mL). After stirring for 8 h, the solvent was evaporated under reduced pressure and the resulting residue was purified by silica gel column chromatography (10% MeOH/DCM) to give **20** as a colorless oil (123 mg, 73%): **^{1}H NMR** (300 MHz, CD_3OD) *δ* 1.76-1.85 (m, 2H, $CH_2CH_2CH_2$), 2.80 (dd, *J* = 14.9, 3.8 Hz, 1H, CH*H*CHO), 3.18-3.32 (m, 3 H, C*H*$_2$NH, C*H*HCHO), 3.43-3.85 (m, 7H, CH-2$_{gal}$, CH-3$_{gal}$, CH-5$_{gal}$, CH*H*CH$_2$CH$_2$NH, CH_2-6$_{gal}$, CH-4$_{gal}$), 3.93-4.00 (m, 1H, C*H*HCH$_2$CH$_2$NH), 4.23 (dd, *J* = 7.4, 2.7 Hz, 1H, CH-1$_{gal}$), 5.43 (s, 1H, CH$_2$C*H*O), 7.29-7.41 (m, 7H, CH-aryl), 7.57 (d, *J* = 7.4 Hz, 1H, CH-aryl); **^{13}C NMR** (75.5 MHz, CD_3OD) *δ* 30.84 ($CH_2CH_2CH_2NH$), 38.92 (CH_2NH), 47.17 (CH_2CHO), 62.53 (CH_2-6$_{gal}$), 68.10 (*C*H$_2$CH$_2$CH$_2$NH), 70.29 (CH-4$_{gal}$), 72.55 (CH-2$_{gal}$), 74.98 (CH-3$_{gal}$), 76.63 (CH-5$_{gal}$), 77.86 (CH_2CHO), 104.95 (CH-1$_{gal}$), 110.99 (*C*≡C), 113.84

(C≡*C*), 122.37 (C-aryl), 124.94 (CH-aryl, C-aryl), 126.87 (CH-aryl), 127.15 (CH-aryl), 128.22 (CH-aryl), 128.28 (CH-aryl), 129.25 (CH-aryl), 129.31 (CH-aryl), 131.05 (CH-aryl), 152.42 (C-aryl), 153.65 (C-aryl), 157.98 (C=O); **MS** (MALDI- ToF) 506.2457 ($C_{26}H_{29}NO_8Na$ ($M+Na^+$) requires 506.1785).

Amino PEG Coumarin derivative 22. Coumarin 343 (100 mg, 0.35 mmol, 1 equiv), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) (273 mg, 0.53 mmol, 1.5 equiv) and *N,N*-diisopropylethylamine (DIPEA) (182 µL, 1.05 mmol, 3 equiv) were dissolved in CH_2Cl_2 (5 mL). The resulting mixture was added dropwise to a stirred solution of 2,2'-(ethylenedioxy)bis-(ethylamine) (511 µl, 3.5 mmol, 10 equiv) in CH_2Cl_2 (5 mL) over a period of 5 min. After stirring for 4 h, the solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography on silica gel (gradient $MeOH/CH_2Cl_2/NH_4OH$ 8/91/1, v/v/v to $MeOH/CH_2Cl_2/NH_4OH$ 10/89/1, v/v/v) yielding **22** as an orange glass–like solid (104 mg, 72%): **^{1}H NMR** (300 MHz, CD_3OD) δ 1.84-1.98 (m, 4H, 2×NCH_2CH_2), 2.59-2.73 (m, 4H, 2×$N(CH_2)_2CH_2$), 2.79-2.94 (m, 2H, CH_2NH_2), 3.23-3.36 (m, 4H, 2×$NCH_2(CH_2)_2$), 3.60 (t, J = 5.21 Hz, 4H, 2×$NHCH_2CH_2$), 3.68-3.71 (m, 6H, 2×CH_2O, $NHCH_2$), 6.83 (s, 1H, CH-aryl), 8.21 (s, 1H, CH-vinyl); **^{13}C NMR** (75.5 MHz, CD_3OD) δ 20.87 ($N(CH_2)_2CH_2$), 20.99 (NCH_2CH_2), 21.98 (NCH_2CH_2), 28.28 ($N(CH_2)_2CH_2$), 40.51 ($NHCH_2$), 42.13 (CH_2NH_2), 50.66 (NCH_2), 51.18 (NCH_2), 70.63 (CH_2O), 71.38 (CH_2O), 71.55 (2×CH_2O), 106.12 (C), 108.02 (C), 109.09 (C), 121.35 (C), 128.29 (CH-aryl), 148.64 (CH-vinyl), 149.76 (C), 153.62 (C), 163.91 (C=O), 165.38 (C=O); **HRMS** (MALDI-ToF) 416.2057 ($C_{22}H_{30}N_3O_5$ ($M+H^+$) requires 416.2180).

Coumarin-DIBO derivative 23. Coumarin derivative **22** (37.0 mg, 0.09 mmol, 1 equiv), 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester (41

mg, 0.11 mmol, 1.2 equiv) and DIPEA (63 µL, 0.36 mmol, 4 equiv) were dissolved in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 24h and concentrated *in vacuo*. The resulting residue was purified by column chromatography on silica gel (50% acetone in hexanes) to give **23** as a yellow glass-like solid (54 mg, 91%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 1.83-1.98 (m, 4H, 2×NCH_2CH_2), 2.69-2.85 (m, 5H, 2×$N(CH_2)_2CH_2$, CH*H*CHO), 3.11 (d, J = 15 Hz, 1H, C*H*HCHO), 3.22-3.30 (m, 4H, 2×$NCH_2(CH_2)_2$), 3.35-3.46 (m, 2H, $NHCH_2$), 3.54-3.79 (m, 10H, 4×CH_2O, $NHCH_2$), 5.44 (brs, 1H, CH_2CHO), 6.01 (t, J = 5.0 Hz, 1H, NH), 6.93 (s, 1H, CH-aryl), 7.20-7.32 (m, 7H, 7×CH-aryl), 7.50 (d, J = 6.5 Hz, 1H, CH-aryl), 8.58 (s, 1H, CH-vinyl), 9.17 (brs, 1H, NH); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 19.99 ($N(CH_2)_2CH_2$, NCH_2CH_2), 21.00 (NCH_2CH_2), 27.32 ($N(CH_2)_2CH_2$), 39.30 ($NHCH_2$), 41.00 ($NHCH_2$), 46.06 (CH_2CHO), 49.66 (NCH_2), 50.09 (NCH_2), 69.75 (CH_2O), 70.12 (CH_2O), 70.32 (CH_2O), 70.40 (CH_2O), 76.51 (CH_2CHO), 105.51 (C), 108.10 (C), 108.78 (C), 109.90 (C≡C), 112.71 (C≡C), 119.53 (C), 121.04 (C), 123.69 (C), 123.88 (CH), 125.70 (CH), 125.93 (CH), 126.81 (2×CH), 126.89 (CH), 127.84 (CH), 127.91 (CH), 129.92 (CH), 147.97 (CH), 148.03 (C), 151.06 (C=O), 152.19 (C), 152.54 (C), 155.62 (C) 162.95 (C=O), 163.60 (C=O); **HRMS** (MALDI-ToF) 684.2219 ($C_{39}H_{39}N_3O_7Na$ ($M+Na^+$) requires 684.2680).

Triazole 24. Azide **17** (10 mg, 0.03 mmol) was added to a solution of galactose-DIBO derivative **20** (14.3 mg, 0.03 mmol) in methanol (2 mL). The reaction mixture was stirred at room temperature for 2 h. The solution was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel using a mixture of 10% methanol in CH_2Cl_2 to give pure triazole **24** (23 mg, 93%): ^{1}H NMR (500 MHz, CD_3OD) δ 1.74 (m, 2H, CH_2), 2.90-3.28 (m, 4H, 2×CH_2), 3.35-4.24 (m, 23H, 8×CH_2, $CHCH_2$, CH_{2gal}, 3×CH_{gal}), 4.50-4.62 (m, 2H,

2×CH_{gal}), 5.85-6.20 (m, 2H, CH_2CHO, NH), 6.80-7.70 (m, 12H, aromH), 8.01 (s, 1H, CH=N); HRMS (MALDI-ToF) 844.3492 ($C_{41}H_{51}N_5O_{13}Na$ (M+Na^+) requires 844.3376).

Isoxazole 25. A methanolic solution (1 mL) of galactose-DIBO derivative **20** (14.3 mg, 0.03 mmol) was added dropwise to a solution of oxime **17** (12.2 mg, 0.036 mmol) and BAIB (11.6 mg, 0.036 mmol) in methanol (1 mL). The reaction mixture was stirred at room temperature for 10 min. The solution was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel using a mixture of 8% methanol in CH_2Cl_2 to give pure isozaxole **25** (14.6 mg, 61%): ^{1}H NMR (500 MHz, CD_3OD) δ 1.70-1.84 (n, 2H, CH_2), 3.30-4.30 (m, 29H, 10×CH_2, CH_2CHOH, CH_{2gal}, 5×CH_{gal}), 6.10-6.40 (m, 1H, CH_2CHOH), 6.70-7.70 (m, 13H, aromH, NH); HRMS (MALDI-ToF) 842.2192 ($C_{41}H_{49}N_5O_{13}Na$ (M+Na^+) requires 842.3219).

Dendron 27. Azido-dendron **26** [15b] (100 mg, 0.061 mmol, 1 equiv) was dissolved in a mixture of THF and H_2O (9:1, v/v, 5 mL). A 1 M solution of trimethylphosphine in THF (0.61 mL, 0.61 mmol, 10 equiv) was added to the solution and the resulting mixture was stirred for 3 h. The solution was concentrated *in vacuo* and coevaporated with toluene (3×10 mL). The resulting residue was purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) to give **27** as a colorless oil (76 mg, 78%): **^{1}H NMR** (300 MHz, $CDCl_3$) δ 1.22-1.27 (m, 21H, 7×CH_3), 1.97 (t, J = 2.5 Hz, 8H, 8×C≡CH), 2.41-2.56 (m, 32H, 8×CH_2CH_2C≡CH), 2.61-3.00 (m, 4H, CH_2NH_2), 3.37-3.59 (m, 10H, CH_2NH, 4×CH_2O), 4.17 - 4.25 (m, 28H, 14×OCH_2), 6.90 (t, J = 5.2 Hz, 1H, NH); **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ 14.19 (8×CH_2C≡CH), 17.43 (2×CH_3), 17.53 (CH_3), 17.72 (4×CH_3), 33.04 (8×CH_2CH_2C≡CH), 39.45 ($NHCH_2$), 41.21 (CH_2NH_2), 46.23 (CH_2), 46.28 (4×CH_2), 46.64 (2×CH_2), 65.18 (8×OCH_2C), 65.30 (4×OCH_2C), 67.07 (2×OCH_2C), 69.27 (8×C≡CH, CH_2O), 69.59 (CH_2O), 69.94 (CH_2O), 70.07 (CH_2O), 82.22 (8×C≡CH), 171.07

(8×C=O), 171.42 (C=O), 171.46 (2×C=O), 171.84 (4×C=O); **MS** (MALDI-ToF) 1601.8 ($C_{81}H_{105}N_2O_{31}$ ($M+H^+$) requires 1601.7).

Glycodendron 28. Dendron **27** (40 mg, 0.025 mmol, 1 equiv), 3-azidopropyl β-D-galactopyranoside (78.6mg, 0.300 mmol, 12 equiv) and tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (10.6mg, 0.020mmol, 0.8 equiv) were dissolved in THF (2 mL). An aqueous solution of (+)-sodium L-ascorbate (0.1 M, 0.6 mL, 0.060 mmol, 2.4 equiv) and $CuSO_4$ (0.1 M, 0.2 mL, 0.020 mmol, 0.8 equiv) were added, and the resulting solution was stirred for 18 h. Solvents were evaporated under reduced pressure and the residue was purified *via* RP-HPLC (0-5min 0% B, 5-30 min gradient of 0-100% B, t=20.8 min). Fractions of interest were combined and lyophilized to give pure **28** as TFA salt (65mg, 70%): **^{1}H NMR** (500 MHz, D_2O) δ 1.12 (s, 12H, 4×CH_3), 1.23 (s, 6H, 2×CH_3), 1.30 (s, 3H, CH_3), 2.20-2.22 (m, 16H, 8×$CH_2CH_2CH_2$-triazole), 2.77-2.80 (m, 16H, 8×CH_2CH_2-triazole), 3.03 (brs, 16H, 8×CH_2CH_2-triazole), 3.20 (t, J = 4.9 Hz, 2H, CH_2NH_2), 3.41 (t, J = 5.3 Hz, 2H, CH_2NH), 3.50 (dd, J = 9.8, 7.9 Hz, 8H, 8×CH-2_{gal}), 3.57-3.77 (m, 48H, 4×CH_2O, 8×CH-3_{gal}, 8×CH-5_{gal}, 8×CH_2-6_{gal}, 8×CHCH_2CH_2-triazole), 3.87-3.94 (m, 16H, 8×CH-4_{gal}, 8×CHHCH_2CH_2-triazole), 4.12-4.27 (m, 28H, 14×OCH_2), 4.34 (d, J = 7.9Hz, 8H, 8×CH-1_{gal}), 4.57 (t, J = 6.8 Hz, 16H, 8×CH_2-triazole), 8.04 (s, 8H, 8×CH of triazole); **^{13}C NMR** (75.5 MHz, D_2O) δ 16.98 (7×CH_3), 19.61 (8×CH_2CH_2-triazole), 29.53 (8×$CH_2CH_2CH_2$-triazole), 32.57 (8×CH_2CH_2-triazole), 39.13 ($NHCH_2$), 39.25 (CH_2NH), 46.38 (CH_2C), 46.47 (4×CH_2C), 46.76 (2×CH_2C), 48.40 (8×CH_2-triazole), 61.12 (8×CH_2-6 of Gal), 65.82 (8×OCH_2C), 66.34 (8×$CH_2CH_2CH_2$-triazole), 66.56 (4×OCH_2C), 67.11 (2×OCH_2C), 68.76 (8×CH-4_{gal}, CH_2O), 68.98 (CH_2O), 69.66 (2×CH_2O), 70.92 (8×CH-2_{gal}), 72.91 (8×CH-3_{gal}), 75.26 (8×CH-5_{gal}), 102.96 (8×CH-1_{gal}), 125.02 (8×CH of triazole), 145.12 (8×C of

triazole), 162.86 (q, J = 35.4 Hz, CF_3), 173.13 (2×C=O), 173.59 (4×C=O), 173.65 (8×C=O), 174.10 (C=O); **MS** (MALDI-ToF) 3706.5576 ($C_{153}H_{241}N_{26}O_{79}$ ($M+H^+$) requires 3706.5635).

DIBO-glycodendron 29. Glycodendron **28** (65.0 mg, 0.017 mmol, 1 equiv) and 11,12-didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-yl carbonic acid 4-nitrophenyl ester[2] (13.5 mg, 0.035 mmol, 2 equiv) were dissolved in DMF (3 mL). DIPEA (9 µL, 0.051 mmol, 3 equiv) was added to the solution and the resulting mixture was stirred for 24 h. The solvent was evaporated under reduced pressure to give a residue, which was purified by RP-HPLC (0-2min 0% B, 2-5 min gradient of 0-20% B, 5-40 min gradient of 20-55% B, t=27.8 min). Fractions of interest were combined and lyophilized to give **29** as a white powder (43.0 mg, 64%): **^{1}H NMR** (600 MHz, D_2O) δ 1.05-1.18 (m, 21H, 7×CH_3), 2.10-2.19 (m, 16H, 8×$CH_2CH_2CH_2$-triazole), 2.55 (d, J = 14.6 Hz, 1H, CH*H*CHO), 2.70 (t, J = 6.9 Hz, 16H, 8×CH_2CH_2-triazole), 2.95 (t, J = 6.8 Hz, 16H, 8×CH_2CH_2-triazole), 3.09 (d, J = 14.0 Hz, 1H, C*H*HCHO), 3.26-3.33 (m, 4H, 2×CH_2NH), 3.49 (dd, J = 9.6, 8.1 Hz, 8H, 8×CH-2_{gal}), 3.55-3.74 (m, 48H, 4×CH_2O, 8×CH-3_{gal}, 8×CH-5_{gal}, 8×CH_2-6_{gal}, 8×CH*H*CH_2CH_2-triazole), 3.83-3.91 (m, 16H, 8×CH-4_{gal}, 8×C*H*HCH_2CH_2-triazole), 4.01-4.22 (m, 28H, 14×OCH_2), 4.30 (d, J = 7.9Hz, 8H, 8×CH-1_{gal}), 4.50 (t, J = 6.8 Hz, 16H, 8×CH_2-triazole), 5.16 (brs, 1H, CH_2C*H*O), 7.18-7.33 (m, 7H, 7×CH aryl), 7.48 (d, J = 6.8 Hz, 1H, CH aryl), 7.91 (s, 8H, 8×CH of triazole); **^{13}C NMR** (125 MHz, CD_3OD:$CDCl_3$, 4/1, v/v) δ 17.77 (CH_3), 18.03 (2×CH_3), 18.15 (4×CH_3), 21.48 (8×$CH_2CH_2CH_2$-triazole), 31.19 (8×$CH_2CH_2CH_2$-triazole), 34.00 (8×$CH_2CH_2CH_2$-triazole), 40.25 (CH_2NH), 41.44 (CH_2NH), 46.85 (CH_2CHO), 47.27 (CH_2C), 47.32 (4×CH_2C), 47.63 (2×CH_2C), 47.90 (8×CH_2-triazole), 62.14 (8×CH_2-6_{gal}), 66.11 (12×OCH_2C), 66.60 (8×$CH_2CH_2CH_2$-triazole), 67.87 (2×OCH_2C), 69.81 (8×CH-4_{gal}), 70.20 (CH_2O), 70.67 (CH_2O), 70.92 (2×CH_2O), 72.16 (8×CH-2_{gal}), 74.52 (8×CH-3_{gal}), 76.11 (8×CH-5_{gal}), 77.63 (CH_2CHO), 104.47 (8×CH-1_{gal}), 110.54 (C≡C), 113.33 (C≡C),

121.92 (C-aryl), 123.89 (8×CH of triazole), 124.50 (CH-aryl), 124.65 (C-aryl), 126.88 (CH-aryl), 127.15 (CH-aryl), 128.02 (CH-aryl), 128.08 (CH-aryl), 129.11 (2×CH-aryl), 130.81 (CH-aryl), 151.80 (C-aryl), 152.98 (C-aryl), 157.42 (C=O), 146.86 (8×C of triazole), 172.79 (2×C=O), 173.17 (4×C=O), 173.23 (8×C=O), 173.54 (C=O); **MS** (MALDI-ToF) 3974.7 ($C_{170}H_{250}N_{26}O_{81}Na$ ($M+Na^+$) requires 3974.6).

General Procedure for SPAAC with Bi-functional Linker 17. Bi-functional linker **17** (0.03 mmol, 10.1 mg) and corresponding DIBO derivative **30** or **23** (0.03 mmol) were dissolved in MeOH or THF (in case of coumarin-DIBO derivative **23**) (2 mL). The reaction mixture was stirred for 3 h and the solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel.

Triazole 31. Purification by silica gel column chromatography (5 then 10% MeOH in CH_2Cl_2) gave **31** as a colorless oil (25.1 mg, 87%): ^{1}H NMR (300 MHz, CD_3OD) δ 1.34-1.45 (m, 2H, $CHCH_2CH_2$), 1.52-1.76 (m, 4H, $CHCH_2CH_2CH_2$), 2.15-2.21 (m, 2H, CH_2C=O), 2.64-2.69 (m, 1H, CHHS), 2.85-3.74 (m, 26H, CHHS, 9×CH_2O, 2×CH_2NH, CH_2CHO, CHS), 3.83-4.06 (m, 4H, 2×CH_2O), 4.21-4.28 (m, 1H, CHNH), 4.41-4.47 (m, 1H, CHNH), 4.55-4.61 (m, 2H, CH_2-triazole), 5.89-6.17 (m, 1H, CH_2CHO), 6.83-6.88 (m, 2H, aromH), 7.15-7.65 (m, 10H, aromH), 8.01 (s, 1H, CH=N); MS (MALDI-ToF) 981.4092 ($C_{46}H_{62}N_8O_{11}SNa$ ($M+Na^+$) requires 981.4157).

Triazole 32. Purification by silica gel column chromatography (3% MeOH in CH_2Cl_2) gave **32** as a yellow amorphous solid (22 mg, 73%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80-2.02 (m, 4H, 2×$NCH_2CH_2CH_2$), 2.66-2.86 (m, 4H, 2×$NCH_2CH_2CH_2$), 3.01-4.12 (m, 32H, 2×$NCH_2CH_2CH_2$, 11×CH_2O, 2×CH_2NH, CH_2CHO), 4.37-4.61 (m, 2H, CH_2-triazole), 5.34-6.49 (m, 2H, CH_2CHO, NH), 6.73-6.82 (m, 2H, aromH), 6.93-7.60 (m, 11H, aromH), 7.92-8.10 (m,

1H, NH), 8.56-8.68 (m, 1H, CH=N), 9.01-9.25 (m, 1H, CH-vinyl); MS (MALDI-ToF) 1022.4133 ($C_{54}H_{61}N_7O_{12}Na$ ($M+Na^+$) requires 1022.4270).

General Procedure for SPANOC between Triazoles 31 or 32 and Glycodendrimer 29. To a stirred solution of DIBO-glycodendrimer **29** (20.5 mg, 5.2 μmol) and oxime **31** or **32** (5.2 μmol) in MeOH/CH_2Cl_2 (3/1, v/v, 1.2 ml) was added a solution of BAIB (1.8 mg, 5.7 μmol) in MeOH (0.18 mL) and the reaction mixture was stirred for 30 min. The solvent was evaporated and the residue was purified by RP-HPLC. Appropriate fractions were combined and lyophilized.

Glycodendrimer-Biotin Conjugate 33. After RP-HPLC purification (0-5min 0% B, 5-40 min gradient of 0-100% B, t=29.4 min) and lyophilization, **33** was obtained as a white powder (14.0 mg, 55%): ^{1}H NMR (500 MHz, D_2O) δ 0.88-1.22 (m, 23H, 7×CH_3, CHCH$_2$C*H*$_2$), 1.32-1.63 (m, 4H, CHC*H*$_2$CH$_2$C*H*$_2$), 1.95-2.22 (m, 18H, 8×CH$_2$C*H*$_2$CH$_2$-triazole, C*H*$_2$C=O), 2.48-2.80 (m, 17H, CH*H*S, 8×C*H*$_2$CH$_2$-triazole), 2.80-3.02 (m, 17H, C*H*HS, 8×CH$_2$C*H*$_2$-triazole), 3.08-3.95 (m, 107H, 2×CH$_2$CHO, 4×C*H*$_2$NH, 15×CH$_2$O, 8×CH-2$_{gal}$, 8×CH-3$_{gal}$, 8×CH-5$_{gal}$, 8×CH$_2$-6$_{gal}$, 8×CH-4$_{gal}$, 8×C*H*$_2$CH$_2$CH$_2$-triazole, CHS), 3.99-4.55 (m, 56H, 9×CH$_2$-triazole, 14×OCH$_2$, 2×C*H*NH, 8×CH-1$_{gal}$), 5.55-6.15 (m, 2H, 2×CH$_2$C*H*O), 6.33-7.60 (m, 20H, aromH), 7.87 (s, 8H, 8×CH$_{triazole}$); MS (MALDI-ToF) 4933.4 ($C_{218}H_{310}N_{34}O_{92}SNa$ ($M+Na^+$) requires 4933.0).

Glycodendrimer-Coumarin Conjugate 34. After RP-HPLC purification (0-5min 0% B, 5-10 min gradient of 0-40% B, 10-30 min gradient of 40-60% B, t=25.3 min) and lyophilization, **34** was obtained as a yellow powder (15.1 mg, 61%): ^{1}H NMR (500 MHz, D_2O:CD_3CN, 1:1, v/v) δ 0.99-1.20 (m, 21H, 7×CH_3), 1.65-1.81 (m, 4H, 2×NCH$_2$C*H*$_2$CH$_2$), 1.99-2.08 (m, 16H, 8×CH$_2$C*H*$_2$CH$_2$-triazole), 2.59-2.62 (m, 20H, 2×NCH$_2$CH$_2$C*H*$_2$, 8×C*H*$_2$CH$_2$-triazole), 2.84 (t, *J* =

7.3 Hz, 16H, 8×CH_2CH_2-triazole), 3.10-3.94 (m, 110H, 2×CH_2CHO, 4×CH_2NH, 15×CH_2O, 2×$NCH_2CH_2CH_2$, 8×CH-2_{gal}, 8×CH-3_{gal}, 8×CH-5_{gal}, 8×CH_2-6_{gal}, 8×CH-4_{gal}, 8×$CH_2CH_2CH_2$-triazole), 3.94-4.25 (m, 36H, 14×OCH_2, 8×CH-1_{gal}), 4.25-4.45 (m, 18H, 9×CH_2-triazole), 5.41-6.19 (m, 2H, 2×CH_2CHO), 6.58-7.51 (m, 21H, aromH), 7.64 (s, 8H, 8×$CH_{triazole}$), 8.36-9.12 (m, 1H, CH-vinyl); MS (MALDI-ToF) 4972.8 ($C_{224}H_{309}N_{33}O_{93}Na$ ($M+Na^+$) requires 4974.0).

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