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INVITED REVIEW

Peptide and glycopeptide dendrimers and analogous dendrimeric structures and their biomedical applications

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Abstract The size of information that can be stored in nucleic acids, proteins, and carbohydrates was calculated. The number of hexamers for peptides is 64,000,000 (20^6) and seems to be impressive in comparison with 4,096 (4^6) hexanucleotides, but the number of isomers of hexasaccharides is 1.44×10^{15}. Carbohydrates are therefore the best high-density coding system. This language has been named glycocode resp. sugar code. In comparison with peptide dendrimers, the amount of information carried by glycopeptide dendrimers or glycodendrimers is therefore much higher. This is reflected by the variability of structures and functions (activities). This review is about the broad area of peptide and glycopeptide dendrimers. The dendrimeric state and physicochemical properties and general consequences are described, together with a cluster effect. The impact of cluster effect to biological, chemical, and physical properties is discussed. Synthesis of dendrimers by convergent and divergent approaches, "Lego" chemistry, ligation strategies, and click chemistry is given with many examples. Purification and characterization of dendrimers by chromatographic methods, electromigration methods, and mass spectrometry are briefly mentioned. Different types of dendrimers with cyclic core, i.e. RAFTs, TASPs and analogous cyclic structures, carbopeptides, carboproteins, octopus glycosides, inositol-based dendrimers, cyclodextrins, calix[4]arenes, resorcarenes, cavitands, and porphyrins are given. Dendrimers can be used for creation of libraries, catalysts, and solubilizing agents. Biocompatibility and toxicity of dendrimers is discussed, as well as their applications in nanoscience, nanotechnology, drug delivery, and gene delivery. Carbohydrate interactions of glycopeptide dendrimers (bacteria, viruses, and cancer) are described. Examples of dendrimers as antiprion agents are given. Dendrimers represent a fast developing area which partly overlaps with nanoparticles and nanotechnologies.

Keywords Calixarene dendrimers · Calix[4]resorcarene dendrimers · Carbopeptide dendrimers · Contrast agents · Cyclodextrin dendrimers · Dendrimers · Drug delivery · Glycobiology · Glycocluster · Glycoconjugates · Glycodendrimers · Glycopeptide dendrimers · Glycopeptide libraries · Glycopeptides · Glycotope · Lectin · Imaging agents · Ligation chemistry · Multiple antigen glycopeptide (MAG)

Dedicated to Miroslav Ledvina, Ph.D. on the occasion of his 60th birthday.

Standard abbreviations have been followed throughout this paper (J Peptide Sci 12:1–12, 2006). When not stated otherwise, amino acids are of L-configuration and carbohydrates are of D-configuration.

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Abbreviations

ACE	Affinity capillary electrophoresis
AChE	Acetylcholinesterase
AFM	Atomic force microscopy
AMINAP	6-Aminomethyl-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene
ATRP	Atom transfer radical polymerization

BINAP	2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene
BNNTs	Boron nitride nanotubes
Carbo-BINAP	2,2′-Bis(diphenylphosphino)-1,1′-binaphthalene-6-carboxylic acid
CCIs	Carbohydrate–carbohydrate interactions
CD	Circular dichroism, or Cyclodextrin
CE	Capillary electrophoresis
CNTs	Carbon nanotubes
ConA	*Canavalia ensiformis* agglutinin; Concanavalin A
CSP	Chiral stationary phase
CZE	Capillary zone electrophoresis
DC	Dendritic cell
DCL	Dynamic combinatorial library
DCR	Dendritic chain reaction
DFT	Density functional theory
Dns	Dansyl
DTPA	Diethylenetriaminepentaacetic acid
ELLA	Enzyme-linked lectin assay
EM	Electrophoretic mobility
EPO	Erythropoietin
EPR	Electron paramagnetic resonance
FimH	A protein found at the tip of a bacterial pilus which adheres to the urinary bladder
FITC	5-Fluorescein isothiocyanate
FTICR-MS	Fourier transform-ion cyclotron resonance-MS
Gd-DTPA	Gd(III)-diethylenetriaminepentaacetic acid
GM3	(*N*-Acetylneuraminyl)-galactosylglucosylceramide
GNA	*Galanthus nivalis* agglutinin from the snowdrop bulb
GNPs	Gold glyconanoparticles
HPA	*Helix pomatia* agglutinin
HRPO	Horseradish peroxidase
IL-1β	Interleukin-1-β
INF-γ	Interferon-γ
ITC	Isothermal titration calorimetry
Le^a	Galβ3(Fucα4)GlcNAcβ3Galβ4Glc
Le^x	Galβ4(Fucα3)GlcNAcβ3Galβ4Glc
MAG	Multiple antigen glycopeptide
MD	Molecular dynamics
MRI	Magnetic resonance imaging
NAC	*N*-alkyl cysteine
NKR	Natural killer cell receptor
PAGE	Polyacrylamide gel electrophoresis
PAMAM	Polyamidoamine Starburst dendrimer
PAMAM-SAH	PAMAM succinamic acid dendrimers
PEC	Pentaerythritol core
PEG	Polyethylene glycol
PEGA	Polyethylene glycol polyacrylamide resin
PEI	Poly(ethyleneimines)
PePO	Propargylated pentaerythritol phosphodiester oligomer
PNA	*Arachis hypogaea* agglutinin
POEPOP	Polyoxyethylene–polyoxypropylene resin
PPI	Poly(propyleneimine)
PSP	Pseudostationary phase
PTA	*Psophocarpus tetragonolobus* agglutinin
PXM	Piroxicam
QqTOF-MS	Quadrupole–quadrupole time-of-flight mass spectrometry
RAFT	Regioselectively addressable functional template
Rho	Rhodamine
RIP	Relative inhibitory potency
SAL	Sugar-assisted ligation
SAMs	Self-assembled monolayers
SDS	Sodium dodecylsulphate
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
Sle^x	Neolactoseries antigens sialyl-Lewis x
SPGS	Solid phase glycopeptide synthesis
SPOCC	Superpermeable organic combinatorial chemistry resin
SPPS	Solid phase peptide synthesis
SPR	Surface plasmon resonance
SRCD	Synchrotron radiation-based circular dichroism
STM	Scanning tunneling microscopy
SWNTs	Single-walled carbon nanotubes
TASP	Template-assembled synthetic protein
TEM	Transmission electron microscopy
TentaGel	PEG-grafted resin
TF antigen	Galβ(1 → 3)GalNAcα(1 → O)Ser/Thr
T_N antigen	GalNAcα(1 → O)Ser/Thr
TNF-α	Tumor necrosis factor-α
UPLC	Ultra performance liquid chromatography
WGA	Wheat germ (*Triticum vulgaris*) agglutinin

Introduction

Over 13,100 published references (Web of Science, 26 April 2010) in the dendrimer field clearly show the merits

and perspectives of this class of compounds (Tomalia 2009). Presently, there are over 1,000 patents dealing with dendritic structures (Carlmark et al. 2009; Niederhafner et al. 2008b) and about 1,000 papers and 150 patents are published annually (Niederhafner et al. 2008b). Dendrimers are successfully expanding to all areas of bioorganic chemistry, biological and medical applications, nanotechnology, and many other fields. We can say that the area of dendrimers is fastly dendrimering (Niederhafner et al. 2008b).

This review is a free continuation of our reviews about glycopeptide dendrimers (Niederhafner et al. 2008a, b; c) and peptide dendrimers (Niederhafner et al. 2005). Therefore, for the explanation of basic terms like nomenclature of dendrimers, classes of glycopeptide dendrimers, dendriplexes and dendrisomes, dendrigraft, glycotope, "smart" glycodendrimers, minicluster (microcluster) and macrocluster (maxicluster), multiple antigen glycopeptides (MAGs) and their classification, poly (propyleneimine) dendrimers, PAMAM dendrimers, aminobis (polypropylamine) MAGs, linear polymers (oligomers) with variable valency (brush dendrimers, comb dendrimers), sequential oligopeptide carriers, chitosan-based dendrimers, self-immolative dendrimers (cascade release dendrimers, domino dendrimers), microwave-assisted synthesis of dendrimers, chirality of dendrimers, etc. the reader is referred to these reviews. We included mainly glyco- and glycopeptide dendrimers. Because some synthetic principles, physicochemical properties, and biological and biomedical applications are common for different types of dendrimers, they are included too. Therefore, the other types of dendrimers (e.g. without sugar) are given as examples and inspirative hints, usable also in the glycopeptide dendrimer field. The unifying idea in the dendrimer field is the cluster effect. Our approach to dendrimers is not strictly limited to regular geometrical branching, but more generally to branched compounds, where the cluster effect plays an important role. The reason is simple: they can be applicable in the area of glycopeptide dendrimers.

The first paper about glycopeptide dendrimers appeared in 1993 (Roy et al. 1993). The sugar attached was sialic acid that was introduced to confer strong inhibitory properties against flu virus hemagglutinin, a lectin-like protein recognizing α-sialosides on respiratory mucins.

The history of dendrimers has been described in detail (Frauenrath 2005; Svenson and Tomalia 2005; Rosen et al. 2009).

The literature about glycopeptide dendrimers and dendrimers in general is growing very fast. Many review articles appeared during the last few years (Chabre and Roy 2010; Astruc et al. 2010; Meldal et al. 2010; Heegaard et al. 2010; Dutta et al. 2010; Rosen et al. 2009; Tomalia 2009; Svenson 2009; Rolland et al. 2009; Labieniec and Watala 2009; Nanjwade et al. 2009; Rojo 2009; Gabius 2009; Paleos et al. 2009; Scholl et al. 2009; Tekade et al. 2009b; Bharali et al. 2009; Falciani et al. 2009; Mishra et al. 2009; Yellepeddi et al. 2009; Wang and Kaifer 2009; Arima and Motoyama 2009; Li and Aida 2009; Maes and Dehaen 2009; Martini and Ciani 2009; van Dongen et al. 2009; Shcharbin et al. 2009; Biricova and Laznickova 2009; Fako and Furgeson 2009; Carlmark et al. 2009; Muthu and Singh 2009; Leung et al. 2009; Chabre and Roy 2008; Pini et al. 2008; Svenson and Chauhan 2008; Niederhafner et al. 2008a, b, c; Imberty et al. 2008; Newkome and Shreiner 2008; Paleos et al. 2008; Cheng et al. 2008b; Toth et al. 2008; Renaudet 2008; Villalonga-Barber et al. 2008; Wolinsky and Grinstaff 2008; Theodossiou et al. 2008; Chadha et al. 2008; Hein et al. 2008; Mourya and Inamdar 2008; Martos et al. 2008; Roy and Touaibia 2007; Touaibia and Roy 2007; Svenson 2007; Rojo and Delgado 2007; Heegaard et al. 2007; Balogh 2007; Pieters et al. 2007; Paleos et al. 2007; Dondoni 2007; Kehat et al. 2007; Li et al. 2007; Crampton and Simanek 2007; Boas et al. 2006a, b, c, d, e, f, g; Svenson and Tomalia 2006; Heegaard and Boas 2006; Fernandez et al. 2006; Bai et al. 2006; Portney and Ozkan 2006; Gupta et al. 2006; Schalley et al. 2006; Darbre and Reymond 2006; Smith 2006; Doores et al. 2006; Yang and Kao 2006; Svenson and Tomalia 2005; Caminade et al. 2005; Tomalia 2005a; Niederhafner et al. 2005; Tsvetkov and Nifantiev 2005; Rosa Borges and Schengrund 2005; Shi et al. 2005b; Duncan and Izzo 2005; Smith et al. 2005; Frauenrath 2005; Lockman et al. 2005; Lee et al. 2005a; Gillies and Frechet 2005; Liang and Frechet 2005; Jianga and Aida 2005; Dufes et al. 2005; Boas and Heegaard 2004; Patri et al. 2005; Roy 2003; Tomalia and Majoros 2003; Bezouska 2002; Lundquist and Toone 2002).

Dendrimers can be applied in many areas, e.g. as *anticancer polymeric nanomedicines and nanocarriers* (Astruc et al. 2010; Rosen et al. 2009; Rolland et al. 2009; van Dongen et al. 2009; Fox et al. 2009; Portney and Ozkan 2006; Yellepeddi et al. 2009; Chadha et al. 2008; Svenson and Tomalia 2005, 2006; Tong and Cheng 2007; Bai et al. 2006; Paleos et al. 2007, 2008; Cheng et al. 2007, 2008a, b; Falciani et al. 2009; Emerich and Thanos 2008; Hamilton and Harth 2009; Cheng and Xu 2008; Bharali et al. 2009; Svenson 2009; Tekade et al. 2009b); *drug delivery* (Astruc et al. 2010; Rosen et al. 2009; Rolland et al. 2009; Labieniec and Watala 2009; van Dongen et al. 2009; Shcharbin et al. 2009; Martini and Ciani 2009; Yellepeddi et al. 2009; Tekade et al. 2009b; Arima and Motoyama 2009; Yoon and Jang 2010; Nanjwade et al. 2009; Fox et al. 2009; Theodossiou et al. 2008; Muthu and Singh 2009; Pini et al. 2008; Mourya and Inamdar 2008; Jain 2008b; Chadha et al. 2008; Rojo and Delgado 2007; Balogh 2007; Li and Loh 2008; Scholl et al. 2009; Bharali

et al. 2009; Renaudet 2008; Jain 2008a; Falciani et al. 2009; Dutta et al. 2010; Boas et al. 2006b, c, d; Darbre and Reymond 2006; Portney and Ozkan 2006; Svenson and Tomalia 2006; Crampton and Simanek 2007; Svenson 2007, 2009; Villalonga-Barber et al. 2008; Yang and Kao 2006; Lee et al. 2005a; Doores et al. 2006; Paleos et al. 2007, 2009; Kehat et al. 2007; Gupta et al. 2006; Heegaard and Boas 2006; Bai et al. 2006; Cheng and Xu 2008; Cheng et al. 2008b; Paleos et al. 2008; Majoros et al. 2008; Rosa Borges and Schengrund 2005; Gillies and Frechet 2005; Duncan and Izzo 2005; Dufes et al. 2005; Svenson and Tomalia 2005; Patri et al. 2005; Boas and Heegaard 2004; Svenson and Chauhan 2008; Emerich and Thanos 2008; Hamilton and Harth 2009; Cheng et al. 2007; Cheng et al. 2008a); *gene carriers and vectors in gene delivery* (Arima and Motoyama 2009; Rosen et al. 2009; Rolland et al. 2009; van Dongen et al. 2009; Paleos et al. 2008; Shcharbin et al. 2009; Dutta et al. 2010; Yellepeddi et al. 2009; Nanjwade et al. 2009; Duncan and Izzo 2005; Paleos et al. 2007, 2009; Crampton and Simanek 2007; Dufes et al. 2005; Svenson 2007; Tekade et al. 2009b); *cancer diagnosis and therapy* (Astruc et al. 2010; Rolland et al. 2009; Tekade et al. 2009b; Falciani et al. 2009; Pini et al. 2008; Chadha et al. 2008; Bharali et al. 2009; Villalonga-Barber et al. 2008; Paleos et al. 2009; Svenson and Tomalia 2006; Svenson 2007, 2009; Renaudet 2008; Portney and Ozkan 2006; Li and Loh 2008; Bezouska 2002; Toth et al. 2008; Crampton and Simanek 2007; Svenson and Chauhan 2008; Boas et al. 2006e; Majoros et al. 2008; Wolinsky and Grinstaff 2008; Emerich and Thanos 2008; Cheng et al. 2007, 2008a, b; Cheng and Xu 2008; Tomalia et al. 2007; Gupta et al. 2006; Patri et al. 2005; Khan et al. 2005; Svenson and Tomalia 2005; Gillies and Frechet 2005; Bai et al. 2006; Darbre and Reymond 2006; Niederhafner et al. 2008c); *contrast agents for molecular imaging* (Astruc et al. 2010; Rosen et al. 2009; Rolland et al. 2009; Tekade et al. 2009b; van Dongen et al. 2009; Portney and Ozkan 2006; Mody et al. 2009; Kobayashi and Brechbiel 2005; Longmire et al. 2008; Svenson 2007; Tomalia et al. 2007; Majoros et al. 2008; Wolinsky and Grinstaff 2008; Svenson and Tomalia 2005; Bai et al. 2006; Khan et al. 2005).

Peptide and glycopeptide dendrimers can also serve as *immune response modulators* (Heegaard et al. 2010; Zhong et al. 2009; Toth et al. 2008; Rojo 2009; Chabre and Roy 2008; Heegaard and Boas 2006); *vaccines against infectious diseases and cancer* (Heegaard et al. 2010; Zhong et al. 2009; Toth et al. 2008; Villalonga-Barber et al. 2008; Renaudet 2008; Rojo and Delgado 2007; Rojo 2009; Chabre and Roy 2008; Niederhafner et al. 2008c; Martos et al. 2008; Kehat et al. 2007; Roy and Touaibia 2007; Doores et al. 2006; Heegaard and Boas 2006; Tsvetkov and Nifantiev 2005; Boas and Heegaard 2004); and *dendrimer based anti-infective and anti-inflammatory drugs* (Rolland et al. 2009; Roy and Touaibia 2007; Rojo and Delgado 2007; Rosa Borges and Schengrund 2005; Heegaard and Boas 2006; Cheng and Xu 2008; Pini et al. 2008; Cheng et al. 2008a, b; Renaudet 2008; Villalonga-Barber et al. 2008; Svenson 2007, 2009; Svenson and Chauhan 2008; Svenson and Tomalia 2006; Chabre and Roy 2008; Boas et al. 2006e; Boas and Heegaard 2004; Niederhafner et al. 2008c).

The highly branched, multivalent nature and molecular architecture of dendrimers makes them *ideal tools for a variety of tissue engineering applications* (Joshi and Grinstaff 2008; Scholl et al. 2009; Martos et al. 2008), including crosslinking agents, modulators of surface charge and surface chemistry, and as primary components in scaffolds that mimic natural extracellular matrices.

Sugar code

Peptide and phosphodiester bonds commonly lead to a linear structure defined completely by the sequence. Its permutations are the only source of coding capacity. This situation is absolutely different for carbohydrates (Gabius 2008, 2009; Gabius et al. 2004). Four additional parameters in glycans dramatically increase their coding capacity: (1) positions of linkage points (e.g. 1 → 2, 1 → 3, 1 → 4 or 1 → 6); (2) anomeric position (α or β; glycogen/starch in comparison with cellulose differs only in this parameter); (3) ring size (pyranose or furanose); and (4) introduction of branches (Gabius 2008; Gabius et al. 2004). In order to characterize the structure of any saccharide completely, the sequence and all listed parameters (first and second dimensions of the sugar code) have to be defined. α-Lactose is thus not simply galactosyl–glucose (Gal–Glc), but β-Gal*p*-(1 → 4)-α-Glc*p*. More work is needed for structural determination of saccharides than for corresponding nucleotides or peptides. Therefore, breaking of the sugar code is delayed behind deciphering the other two coding systems (Gabius 2008; Gabius et al. 2004). These factors generate a diversity (Gabius 2008, 2009; Gabius et al. 2004) that marks carbohydrates widely off from proteins or nucleic acids.

The size of information that can be stored in nucleic acids, proteins, and carbohydrates was calculated (Gabius et al. 2002, 2004; Laine 1997; Gabius 2008, 2009; Niederhafner et al. 2008a). For trimers of nucleotides using the four pyrimidine and purine bases there are 64 sequence permutations (4^3). In case of peptide, there are the standard 20 amino acids which can form 8,000 isomers (20^3). Under the same conditions, sugars possess 9,000,000 isomers. Branching of saccharides must be reflected in the pool size of oligomers larger than trimers. The number of hexamers for peptides is 64,000,000 (20^6) and seems to be impressive

in comparison with 4,096 (4^6) hexanucleotides, but the number of isomers of hexasaccharides is 1.44×10^{15}. Carbohydrates are therefore the best high-density coding system. This language has been named glycocode resp. sugar code (Solis et al. 2001; Ambrosi et al. 2005), representing the potential level of complex information that carbohydrate structures are able to convey. Monosaccharides, the building units for oligo- and polysaccharides, constitute therefore high-capacity information-storing coding units, the third alphabet of life. In comparison with peptide dendrimers, the amount of information carried by glycopeptide dendrimers or glycodendrimers is therefore much higher, including their structural variability, complexity, spectrum of biological activities, etc.

Some authors use the term sugar code (Gabius 2008, 2009; Gabius et al. 2004), others use glycocode (Ambrosi et al. 2005; Niederhafner et al. 2008a).

Scope and limitations

The choice how to organize the review was difficult (Niederhafner et al. 2008a). As chemists, we decided to order it in accordance with dendrimer physicochemical properties, synthetic pathways (convergent, divergent), and their structure. As a consequence, important compounds and their activities could be omitted. In order to prevent these losses of important topics, the second part of the review contains applications and biomedical properties of dendrimers. For tumor-associated carbohydrate antigen-derived glycopeptide dendrimers, and anti-HIV and other antiviral constructs see Niederhafner et al. (2008c).

In Table 1, it was more logical to arrange the entries according to their biological activities (drug delivery, lectins, antibacterial activity, etc.). In the table, only some selected examples are given without demand for completeness. In some cases one item could be at more places, e.g. bacteria, lectins, and drug delivery. We will use the term MAGs for branched compounds with sugar on the surface in analogy with MAPs (Tam 2004; Niederhafner et al. 2008a). For other types of compounds, the term glycopeptide dendrimers will be used.

The dendrimeric state

Physicochemical properties and general consequences

There are many definitions of dendrimers. We have chosen only three representative examples. The first one describes dendrimers as monodisperse polymers adopting a globular three-dimensional shape with increase of the generation number (Gn). These highly branched macromolecules have an explicit core, interior region shells, and an exterior grooved surface, providing a high surface area-to-volume ratio. The higher generation of dendrimer, the higher number of end groups, and the properties of the dendrimer become more influenced by the nature of the end groups. Unique chemical and physical properties of dendrimers are given by the above-mentioned chemical and structural attributes (Grinstaff 2002).

The second, more lyrical approach (Schluter and Rabe 2000) defines dendrimers as "a jungle of entangled branches traversed by winding trails which lead to sweet fruits and bright blossoms". Using these trails, the thicket's interior can be approached as well as one can track a way out. In this definition, the thicket represents regularly branched, thickly packed structures, whereas the trails stand for empty space and channels filled by solvent. The fruits and blossoms represent electrochemically, photochemically, or synthetically addressable species. They can also stand for catalytically active sites. The motions to and fro the trails can be viewed as transport processes.

The third one describes dendrimers as core–shell nanostructures with well-defined architecture and low polydispersity, which are synthesized in a shell-by-shell manner (expressed in "generations") around a core unit, providing high level of control over size, branching points, and surface properties (Svenson and Chauhan 2008).

Prior to 1984, three macromolecular architectural classes (i.e. linear, cross-linked, and branched) were widely recognized for construction of rather polydisperse products of different molecular weights (Svenson 2007; Hartmann and Borner 2009; Darbre and Reymond 2006). After that year, the "dendritic state" is recognized as a new, fourth class of polymer architecture, which can be divided to five subclasses: random hyperbranched polymers, dendrigrafts, dendrons, dendrimers, and tecto(dendrimers) or megamers (Svenson 2007; Svenson and Tomalia 2005; Tomalia 2005a; Newkome and Shreiner 2008; Frauenrath 2005; Gillies and Frechet 2005; Niederhafner et al. 2008a; Boas et al. 2006g; Cheng et al. 2008b; Hartmann and Borner 2009). In accord with Tomalia (Tomalia 2005b, 2009; Svenson 2007) the quantized precision of dendrons/dendrimers allows these entities to be viewed as nanoscale monomer type building blocks, suitable for the construction of regio-cross-linked dendrimers referred to as "megamers". In contrast to synthesis of "prior" macromolecules, the synthesis of dendrimers proceeds smoothly to monodisperse, structure-controlled macromolecular systems similar to those observed in nature (Svenson and Tomalia 2005; Tomalia 2005a; Newkome and Shreiner 2008; Niederhafner et al. 2008a; Boas et al. 2006g; Cheng et al. 2008b). Dendrimeric polymers with polydispersities of Mw/Mn $\sim$ 1.0005–1.05 are routinely obtained in multigram to kilogram scale from commercially available

Table 1 Biological activities of peptide and glycopeptide dendrimers and analogous dendrimeric structures

Drug delivery systems	Tekade et al. (2009b), Nanjwade et al. (2009), van Dongen et al. (2009), Jain (2008a), Martini and Ciani (2009), Scholl et al. (2009), Bharali et al. (2009), Falciani et al. (2009), Dutta et al. (2010), Yellepeddi et al. (2009), Paleos et al. (2008), Crampton and Simanek (2007), Samad et al. (2009), Du et al. (2009), Rojo and Delgado (2007), Svenson (2007), Boas et al. (2006d, c), Fox et al. (2009), Kehat et al. (2007), Svenson (2009), Villalonga-Barber et al. (2008), Paleos et al. (2007, 2009), Heegaard and Boas (2006), Svenson and Tomalia (2005), Boas and Heegaard (2004), Svenson and Chauhan (2008), Cheng et al. (2007, 2008a, b), Cheng and Xu (2008), Tong and Cheng (2007), Svenson and Tomalia (2006), Duncan and Izzo (2005), Majoros et al. (2008), Gupta et al. (2006), Bai et al. (2006), Muthu and Singh (2009), Darbre and Reymond (2006), Roy and Touaibia (2007), Lee et al. (2005a), Patri et al. (2005), Portney and Ozkan (2006)
PAMAM dendrimer-mediated solubilization and formulation development followed by in vitro, in vivo assessment of piroxicam (PXM) nanocomposite were studied. Two dendrimer generations (G3 and G4) were used as models. The optimized formulations containing 0.2% w/v of PXM loaded PAMAM dendrimer at pH 7.4 (G3) and (G4) resulted in significant enhancements of PXM solubility approximately by 107- and 222-fold, respectively. The half-life of elimination for the drug encapsulated in the formulation was significantly higher than that of pure drug. Comparison of all data suggested G4-based formulations to be superior to G3 as well as pure PXM	Prajapati et al. (2009)
Specific delivery of doxorubicin to tumor cells was achieved using PEGylated PAMAM dendrimer–doxorubicin conjugates	Zhu et al. (2010)
The assembly of a novel nanoscopic delivery system in a size dimension of 5–10 nm was described. The conjugated dendritic carrier facilitated the rapid cellular uptake of a nanoparticle–peptide conjugate with up to 25 copies of peptidic cargo by NIH 3T3 cells. This established new way for the implementation of protein and oligonucleotide drugs	Hamilton and Harth (2009)
The inclusion complexes of β-CD dendrimers with hexavalent mannosyl ligands with the anticancer drug docetaxel showed high drug solubilization capability	Benito et al. (2004)
A dendrimer nanocluster with folate attached on one end and a fluorescent dye on the other is held together by complementary strands of DNA. It selectively targets cancer cells and docks with folate receptors on the cell surface	Choi and Baker (2005).
The delivery of antimalarial drug chloroquine phosphate by glycoconjugated peptide dendrimer-based nanoparticulate systems was studied	Agrawal et al. (2007)
Mitosis was inhibited by glycopeptide dendrimer conjugates of colchicine. MAGs with 4 or 8 identical glycoside moieties at their surface (β-Glc, α-Gal, α-GalNAc, or lactose), served as drug-delivery devices for colchicine. These MAGs provide a suitable selective vehicle for the delivery of cytotoxic compounds to cancer cells	Lagnoux et al. (2005), Darbre and Reymond (2006)
Glycodendrimeric nanoparticulate carriers of primaquine phosphate were used for liver targeting	Bhadra et al. (2005)

Table 1 continued

Doxorubicin was efficiently delivered into mouse brain by *myo*- or *scyllo*-inositol scaffolds covered by multiple units of guanidine	Maiti et al. (2007)
Hyperbranched polyamidoamines containing β-cyclodextrin were studied as tools for controlled release of anticancer drug. chlorambucil	Zhou et al. (2009)
Models for carbohydrate interactions	Roy and Touaibia (2007), Renaudet (2008)
CCIs of multivalent glycoconjugates based on lactose-functionalized G4 PAMAM dendrimers with Langmuir monolayers containing GM3 were investigated. The interaction of GM3 monolayer with dendrimers is selective in the presence of Ca^{2+}	Seah et al. (2009)
To evaluate whether the way in which biological ligands are presented at the surfaces of nanomaterials would affect their binding to biological targets, polymer vesicles and dextran-coated iron oxide nanoparticles were successfully functionalized with both dendritic and nondendritic displays of mannose, a well-known multivalent ligand. Their binding affinities were compared using the hemagglutination assay. It was found that for both vesicles and nanoparticles the binding of the dendritic mannose functionalized materials was enhanced by 1–2 orders of magnitude relative to the nondendritic system	Martin et al. (2009)
Synthetic carbosilane dendrimers uniformly functionalized with thioglycoside-type sialic acid moieties were tested as potential influenza neuraminidase inhibitors	Sakamoto et al. (2009), Oka et al. (2009)
GNPs were tested in a mouse melanoma model as possible inhibitors of experimental lung metastasis. The antiadhesive activity of lactose GNPs in tumoral metastasis progression in vivo has been stressed	Rojo et al. (2004)
Interactions of carbohydrates with simple cations of biological relevance (Na^+, K^+, Mg^{2+}, and Ca^{2+}) were studied. The studied oligosaccharides prefer direct contact with K^+ over Na^+. These results are important for current hypotheses on glycocalyx functions	Eriksson et al. (2008)
Click multivalent neoglycoconjugates including glycocyclodextrins were studied as synthetic activators in cell adhesion and stimulation of monocyte/macrophage cell lines	Ortega-Munoz et al. (2007)
Interactions with lectins	Gabius et al. (2004), Bezouska (2002), Ambrosi et al. (2005), Roy (2003), Pieters (2004), Sharon (2006), Rudiger and Gabius (2001), Pieters (2007), Roy and Touaibia (2007), Touaibia and Roy (2007), Gabius (2008), Laughlin and Bertozzi (2009), Renaudet (2008), Martos et al. (2008)
CNTs were coated with a biomimetic polymer designed to mimic cell surface mucin glycoproteins. The functionalized CNTs were then bound to cell surfaces via specific carbohydrate receptors. Whereas unmodified CNTs induced cell death, the functionalized CNTs were found to be nontoxic. They interact with HPA lectin	Chen et al. (2006)
Homogeneous bioactive coatings of CNTs by glycodendrimers were used for preparation of biocompatible nanoparticles which recognizes specific lectins such as ConA, PNA, and PTA	Wu et al. (2008)
BNNTs coated with glycodendrimers can bind to lectins ConA and HPA via ligand–receptor interactions, resisting nonspecific binding of irrelevant proteins. BNNTs can also deliver DNA oligomers to the interior of cells with no apparent toxicity	Chen et al. (2009b)

Table 1 continued

The relative affinity per galactose residue of galactose binding lectin *Ricinus communis* agglutinin 120 (RCA 120) for glycoconjugates bearing one or three galactose residues was different by a factor of 23 when measured under IC_{50} conditions or by direct fluorescence reading	Zhang et al. (2009)
Water soluble and lectin-recognizable octopus glycosides were prepared by Huisgen 1,3-cycloaddition reaction of methyl-2,3,4,6-tetra-*O*-propargyl-*β*-galactopyranoside with 2-azidoethyl glycosides of lactose and *N*-acetyllactosamine. Capillary affinity electrophoresis using fluorescence labeled asialoglycans has shown that the binding of a plant lectin RCA_{120} was inhibited by the glycoclusters 400-fold more strongly than with free lactose	Gao et al. (2005)
Glycodendrimers containing 1,2,3-triazole were prepared by click chemistry. The α-Man-containing glycodendrimers were tested for binding affinities of these glycomimetics toward ConA by ELLA and IC_{50} values were determined. Remarkably, the substitution pattern and the distance between the sugars are the more important parameters influencing the binding capabilities of these compounds	Ortega-Munoz et al. (2009), Perez-Balderas et al. (2009)
PAMAM dendrimers containing TF-antigen (valencies 4, 8, 16, and 32) were tested for protein binding properties using peanut lectin from *Arachis hypogaea* and a mouse monoclonal IgG Ab. Conjugates with higher valency generated stronger binding interactions indicating a cluster effect. The inhibition increase was 460, 960, 1,700, and 3,800, respectively, in comparison with monomeric TF-antigen residue	Roy and Baek (2002, 2003), Baek and Roy (2002)
Cu(II)-self-assembling bipyridyl-glycoclusters bearing the T_N-antigen were studied for relative inhibitory potencies against monomeric allyl α-GalNAc using ELLA with asialoglycophorin and horseradish peroxidase-labeled lectin from *Vicia villosa*. Inhibitory properties of the di- and tetravalent bipyridyl clusters were up to 87-fold increased in comparison with the monomer (IC_{50} 158.3 μM). The Cu(II)-complexes were up to 259-fold more active (IC_{50} 0.61 μM), with the octamer showing the highest affinity	Roy and Kim (2003)
The tetramannosyl-RAFT recognized ConA with IC_{50} 62 μM in comparison with methyl α-mannopyranoside (IC_{50} 1.2 mM), respectively	Renaudet and Dumy (2003)
Competitive ELLA was used to measure the affinities of mannose containing, glucose centered glycoclusters toward ConA	Kohn et al. (2004)
PAMAM dendrimers (G1-G6) functionalized by α-mannose have been tested for binding with ConA and with pea lectin. Both proteins bind methyl α-mannose with specificity, ConA has 4-fold higher affinity than pea lectin. Relative affinities of mannose and glucose substituted PAMAM dendrimers with ConA have shown difference in relative activity between glucose functionalized and mannose functionalized dendrimers 14.7 for G4, 15.6 for G5, and 11.4 for G6, respectively	Wolfenden and Cloninger (2005), Schlick et al. (2005), Woller et al. (2003)
The pro-apoptotic, antiproliferative, and cell surface clustering effects of ConA were inhibited by tetra- and hexavalent mannosides	Fortier et al. (2008)
Calix[4 or 8]arene-based glycoconjugates with terminal *N*-acetyl-glucosamine clusters were tested for their capability to bind lectin and to inhibit of erythrocyte agglutination induced by WGA	Consoli et al. (2004)

Table 1 continued

β-CD dendrimers with multivalent (2, 3, 4, 6) mannosyl ligands were studied as lectin binding inhibitors. The binding inhibition of horseradish peroxidase-labeled ConA to yeast mannan by mannosylated β-CD-dendrimers were expressed as IC_{50} (μM) values. The monosubstituted hexavalent β-CD conjugate with one CD molecule had the lowest IC_{50} (10 μM) with relative efficiency (valency-corrected) of 23	Benito et al. (2004)
Trimannosyl-peptide-β-CD dendrimers were studied for ConA binding by ELLA. Addition of 1-adamantyl-carboxylate (AC) leads to a dramatic increase in ConA binding affinity for some compounds. Addition of a suitable AC scavenger, e.g. a trimeric, α,α'-trehalose-based receptor CT3 caused "switching of" of the AC activated samples. These are the first examples of allosteric activation/deactivation of binding and of the multivalent effect	Smiljanic et al. (2006)
Dendrimers with up to eight α-mannose moieties were attached to aluminum oxide chips. Binding of the fluorescent lectins ConA (from the Jack bean seeds) and GNA (Galanthus nivalis agglutinin from the snowdrop bulb) to the glycodendrimer chips was observable in real time. It was possible in a single experiment to observe the multivalency enhancement or cluster effect in the binding event. These dendrimer-fitted chips represent a valuable tool for screening multivalency effects, including kinetic and thermodynamic data	Branderhorst et al. (2008)
Glycodendrimers bearing covalently bound α-mannopyranoside residues onto a hexachlorocyclotriphosphazene core were tested as potential drug candidates for gastrointestinal and urinary tract infections caused by *E. coli*. The whole set of α-mannopyranoside dendrimers with valencies ranging from 6 to 18 units and different epitope spatial arrangements was tested using the well-established tetrameric phytohemagglutinin ConA. Nephelometry was used to study the relative ability of these mannosylated dendrimers to act as cross-linking reagents. The decavalent dendrimer containing alkyne spacer was the fastest and more complete in forming the insoluble cross-linked lattice	Touaibia and Roy (2008)
The binding interactions of glycopeptide–oligonucleotide RAFT conjugate with specific lectins from *Arachis hypogaea* (peanut) were investigated. The interactions were strong and selective. The conjugate does not bind to the nonspecific lectins like ConA	Singh et al. (2005)
Randomized combinatorial library based on TASP with different carbohydrates and amino acids was used to study interactions with lectins	Dulery et al. (2008)
Upper rim thiourea linked tetrapropoxycalix[4]arene glycoconjugates with exposed two or four glucose, galactose and lactose units have been studied by turbidimetric analysis. The tetraglucosyl and tetragalactosyl derivatives specifically bind to ConA and PNA, respectively	Sansone et al. (2003)
Dendritic sialyloligosaccharides containing Neu5Ac-β-(2 → 3)-Gal linkages were tested by competitive ELISA for inhibition of the biotinylated probe from binding to sialoadhesin, a mammalian macrophage sialic acid binding protein. The most active compound was divalent sialoside cellobiosyl-based structure with $IC_{50} = 0.2$ mM. Surprisingly, the tetravalent sialoside dendrimer ($IC_{50} = 2.4$ mM) was less active than the sialoside monomer ($IC_{50} = 1.4$ mM)	Kalovidouris et al. (2003)
Glycocluster conjugates containing a $CD8^+$ epitope of the Melan-A/Mart-1 melanoma antigen were used as potential anticancer vaccine activating DC-SIGN	Srinivas et al. (2007)
Interactions with rat NKR-P1A and NKR-P1B receptors	

Table 1 continued

N-acetyl-glucosamine-coated PAMAM dendrimer modulates antibody formation via natural killer cell activation. These results indicate that $(GlcNAc)_8$-PAMAM induced upregulation of antibody formation is triggered by NK cell stimulation and depends on expressed NKR-P1 isoforms, particularly NKR-P1C	Hulikova et al. (2009)
Comb-like glycodendrimers containing mono-, di-, or tri-T_N clusters showed surprising reactivities with rat NKR-P1A and NKR-P1B receptors. Whereas monomers and dimers of T_N antigen reacted equally with both isoforms of NKR-P1 receptor, the trimer of T_N antigen reacted exclusively with the rat NKR-P1B isoform	Veprek et al. (2006)
Binding and inhibition experiments of PAMAM glycodendrimers and MAGs (with Man and GlcNAc, respectively) with recombinant extracellular ligand-binding domains of the rat NKR-P1A (activating) and NKR-P1B (inhibitory) receptors showed no differences in the binding of monosaccharide ligands. Oligosaccharides and glycodendrimers showed dramatic differences in the binding. NKR-P1A seems to be one of the most important receptors for the glycodendrimers in vitro and in vivo. $(GlcNAc)_4$-PAMAM dendrimers labeled with fluorescein were used to study the in vitro and in vivo fate of GlcNAc coated PAMAM dendrimers in white blood cells, tumors and other tissues	Krist et al. (2004), Plihal et al. (2004)
Prolonged survival and reduction of tumor growth of B16F10 melanoma were achieved by application of $(\beta$-GlcNAc$)_8$-PAMAM dendrimers with affinity to NKR-P1A receptor. The stimulation of an antitumor immune response was proven	Vannuci et al. (2003)
The interactions of lectins with carbohydrates attached on artificial cell surface were studied. The polymers were designed to mimic native cell-surface mucin glycoproteins. This system provides a platform to study cell-surface phenomena with such a chemical control that cannot be achieved using conventional biological tools	Rabuka et al. (2008).
Interactions with galectins	Pieters (2006), Gabius (2008)
Inhibitory capacities (relative potency) and IC_{50} values of Gal, Lac, and LacNAc-β-CD glycoclusters in relation to the univalent inhibitor lactose in different solid-phase assays, interactions with galectin 1, 3, and 7, discrimination between two prototypes and between prototype vs chimera-type galectins are given	Andre et al. (2004b)
G1–G3 MAGs containing lactose with 3,5-di(2-aminoethoxy)benzoic acid in the branches were studied as binding inhibitors of mammalian galectins to glycoproteins, lactose maxiclusters and cell surface glycoconjugates.	Pieters (2004)
Inhibitory effect of sialic acid on β-CD dendrimers containing SLe^x residues as a model for the interaction between E-selectin and leukocytes was studied using SPR	Furuike et al. (2005)
Bacteria	Pieters (2007), Rojo and Delgado (2007), Boas et al. (2006d), McCarthy et al. (2005), Sharon (2006), Imberty et al. (2008), Roy and Touaibia (2007), Touaibia and Roy (2007), Renaudet (2008), Rojo (2009), Chabre and Roy (2010)
Escherichia coli	Touaibia and Roy (2007), Roy and Touaibia (2007)
Chitosan-bound peptide microtubes assayed at concentrations 1.2–6.5 mg mL^{-1}) caused no detectable *E. coli* growth after overnight incubation at 37°C. These results together with the turbidity measurements confirm that chitosan-conjugated microtubes are potent bacteriostatics and bacteriocides	Henricus et al. (2009)
Tri- and hexavalent mannoside clusters with pentaerythritol and triazole linkages were tested as potential inhibitors of type 1 fimbriated bacteria	Touaibia et al. (2007)

Table 1 continued

Octopus glycosides based oligomannoside mimetics were investigated as inhibitors of type 1 fimbriae-mediated adhesion of *E. coli*	Dubber et al. (2006b)
Glycodendrons as oligomannoside mimetics were tested for their potential as inhibitors of type 1 fimbriae-mediated adhesion of *E. coli* by ELISA. The results were interpreted with regard to sugar valency and spacer characteristics	Heidecke and Lindhorst (2007)
Functionalized carbohydrate-centered glycoclusters with thiourea bridges were tested for their anti-adhesive properties in an ELISA with *E. coli*	Sperling et al. (2007)
Nonavalent glycopeptide dendrimers containing methyl α-mannopyranose were screened as inhibitors of the type 1 fimbriae-mediated adhesion using ELISA. Unfortunately, very poor or no inhibitory activities were found	Patel and Lindhorst (2006)
α-Man-dendrimers containing glycerol and glycerol glycol polyether scaffolds were tested for their ability to inhibit mannose-specific adhesion of *E. coli* (recombinant strain HB 101) expressing only type 1 fimbriae on its surface. The di- and tetravalent α-Man-dendrimers had IC_{50} values approximately 10 × lower than MeMan. Their relative IC_{50} (based on MeMan = 1) were 6–13	Boysen et al. (2003)
Pseudomonas aeruginosa	
The binding of fucosylated pentaerythrityl phosphodiester oligomers (PePOs) bearing 4, 6, 8, and 10 L-fucose residues to the fucose-specific *P. aeruginosa* lectin (PA-IIL) was determined through an enzyme-linked lectin amplification competition assay. The IC_{50} values measured are 10–20 times better than for monovalent L-fucose	Morvan et al. (2007)
A library approach was applied for selection of inhibitors preventing biofilm formation of *P. aeruginosa.* Optimization of the structure led to compounds with IC_{50} 0.025 μM i.e. 440-fold enhancement in potency over L-fucose was achieved	Kolomiets et al. (2007), Johansson et al. (2007, 2008), Darbre and Reymond (2008), Kolomiets et al. (2009)
Vibrio cholerae	Pieters (2004)
Galactose-containing dendrimers with long spacer arms inhibit cholera toxin binding comparably as the natural ganglioside GM1 oligosaccharide does	Branderhorst et al. (2007)
Dendrimeric GM1os and GM2os compounds, prepared by "click" chemistry clearly revealed strong multivalent binding to CTB5, with an unparalleled value of at least 380,000-fold stronger binding for octavalent GM1os dendrimer than monovalent GM1os derivatives.	Pukin et al. (2007)
A divalent CT glycocalix[4]arene ligand inhibited CT at low concentration (<200 μM). The efficiency of the divalent compound is superior to that shown by GM1os. Roughly 4,000-fold (2,000-fold per sugar mimic) affinity enhancement for the divalent ligand was achieved. This is exceptionally high in comparison with the one normally measured for a divalent ligand interacting with a polyvalent receptor	Arosio et al. (2005)
Viruses	Rojo (2009), Gajbhiye et al. (2009), Carlescu et al. (2009), Niederhafner et al. (2008c), Rojo and Delgado (2007)
A series of carbosilane dendrimers uniformly functionalized with Neu5Ac-α-(2 → 3)-Gal-β-(1 → 4)-Glc moieties were systematically tested for anti-influenza virus activity. Strong inhibitory activities against human influenza viruses [A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2)] were observed. It led to imporved inhibition activities in the μM level. These compounds are promising therapeutic agents for influenza disease	Oka et al. (2009), Sakamoto et al. (2009)

Table 1 continued

Dendrimers based on cyclotriphosphazene core with phosphonic acid in the branches formed non-covalent complexes with *N*-hexadecylamino lactitol moieties. These supramolecular systems showed submicromolar IC_{50} anti-HIV1 activity	Perez-Anes et al. (2010)
A library containing multivalent water-soluble gold glyconanoparticles (manno-GNPs) presenting truncated $Man_9GlcNAc_2$ was synthesized and tested as inhibitors of DC-SIGN binding to gp120. The best inhibitors of gp120 binding to DC-SIGN are manno-GNPs containing the disaccharide α-Man*p*-(1 → 2)-α-Man*p*. They showed roughly 20,000-fold increase of the activity in comparison with the corresponding monomeric disaccharide	Martinez-Avila et al. (2009)
Dendrimer based imaging and contrast agents	van Dongen et al. (2009), Svenson and Tomalia (2005), Venditto et al. (2005), Portney and Ozkan (2006), Tomalia et al. (2007), Boas et al. (2006e), Choyke and Kobayashi (2006), Kobayashi and Brechbiel (2005), Niederhafner et al. (2008b), Svenson and Tomalia (2006), Tekade et al. (2009b), Longmire et al. (2008), Wolinsky and Grinstaff (2008), Brask et al. (2003b)
Labeled mannosylated MAGs were used to study carbohydrate–protein interactions by fluorescence spectroscopy and imaging methods. FITC-labeled G4 MAG $[Man\text{-}\alpha\text{-}O\text{-}(CH_2)_3CO]_{16}\text{-}K_8K_4K_2KK(FITC)\text{-}CONH_2$ is an excellent probe for the imaging studies of mannose-receptor-mediated entry into dendritic cells by confocal fluorescence microscopy	Kantchev et al. (2008)
DOTA glycopeptide dendrimers (Glc, Gal, Lac) of different valencies (mono, di, and tetra) and their Sm^{3+}, Eu^{3+}, and Gd^{3+} complexes were studied as medical imaging agents. The relaxivity of the Gd^{3+} glycodendrimers and their interaction with the model lectin *Ricinus communis* agglutinin was measured by ^1H nuclear magnetic relaxation dispersion. These glycodendrimers are good candidates for medical imaging agents	Andre et al. (2004a)
Gd-DTPA-terminated PPI dendrimers were evaluated as contrast agents for MRI. The lowest detection concentration of dendrimer was more than two orders of magnitude lower for G5 (8.1×10^{-8} M) than for G0 (3.1×10^{-5} M)	Langereis et al. (2006)
Galactosyl MAGs conjugated with Gd chelates were used as liver-imaging probes. T_1 relaxivity of the dendritic probes was twofold increased to 9.1×10^3 $(Gd\ M)^{-1}\ s^{-1}$ in comparison to Gd-DTPA. These targeting dendrimeric probes were noncytotoxic in vitro and their hepatocyte-cell uptake was much-higher than that of non-targeting ones	Luo et al. (2009)
B16 mouse tumor model system was used for studies of biodistribution of guest–host nanodevices based on gold/PAMAM conjugates. These nanodevices can serve for cancer imaging and therapy	Khan et al. (2005), Nigavekar et al. (2004), Duncan and Izzo (2005)
Artificial viruses	
Calix[4]resorcarene glycocluster nanoparticles were used as model of artificial viruses and their aggregation was studied. Each aggregation strongly depended on the type of the saccharide used for derivatization of the calix[4]resorcarene scaffold. The *β*-Glc viruses were mostly monomeric, α-Glc viruses were highly aggregated and *β*-Gal viruses showed an intermediate oligomeric behavior. The obtained viruses were compactly packed, well charge-shielded and transfect cell cultures (HeLa and HepG2) by a nonspecific but highly size-regulated endocytic pathway, where only monomeric viruses possess substantial transfection activities	Mastrobattista et al. (2006), Aoyama (2004), Boas et al. (2006f), Aoyama et al. (2003), Aoyama (2005), Niederhafner et al. (2008a)

Table 1 continued

CDplexes were studied for their in vitro transfection activity to BNL-CL2 and COS-7 cell lines. The space-oriented dendritic polycationic construct enhancement of gene expression overcame that of polyplexes from commercial PEI polymers (22 kDa)	Diaz-Moscoso et al. (2009)
Self-aggregating (assembling) dendrimers	
A formation of non-covalent nanoparticles by self-assembly of MAGs with 4-64 endgroups Gal-α-(1 → 3)-Gal-β-(1 → 4)-GlcNAc or lactose, both in β-glycosidic form) was studied. These aggregates, in contrast to the individual molecules, efficiently inhibit polyvalent interactions such as IgM binding to the Gal-α-(1 → 3)-Gal-β-(1 → 4)-GlcNAc epitope (αGal), both in vitro and in vivo. Two in vitro assays, the anti-αGal antibody-mediated lysis of pig erythrocytes, and the inhibition of both the anti-αGal IgM binding to the xenoantigen showed highest potency for G2 and G3 (IC_{50} 0.01 μM for both assays), which form large nanoparticles. Therefore, the activity clearly correlates with the size of the aggregates but not with the size of the individual molecules. The most active G3 dendrimer was selected for in vivo profiling in cynomolgus monkeys. Within 5 min after injection, the anti-αGal IgM were reduced to 20% of the initial value. This effect lasted for more than 4 h. The anti-αGal IgM mediated hemolytic activity was completely prevented	Thoma et al. (2006)
Miscellaneous	
Apoptosis-inhibiting proteo-dendrimers with porphyrin core were used as promising candidates for treatment of cancer and hepatitis	Azuma et al. (2009)

chemicals (Svenson and Tomalia 2005; Tomalia 2005a; Newkome and Shreiner 2008; Niederhafner et al. 2008a). This was achieved by simplifications of their synthesis by "Lego"- and "click"-chemistries (Svenson 2007; see "Lego" and "Click chemistry"). As a consequence of multistep dendrimer synthesis, the resulting dendrimer material is always a mixture of both ideal and non-ideal structures (Balogh 2007; Boas et al. 2006g).

Due to the presence of a large number of terminal groups and the limitations or a complete lack of interpenetration, the physicochemical properties of dendrimers are different than those of classical polymers (Balogh 2007; Cheng et al. 2008b). The basic properties of dendrimers are (Tomalia et al. 2007; Bharali et al. 2009; Svenson and Tomalia 2005; Tomalia 2005a, 2009; Svenson 2007, 2009):

1. The dimensions in nanometer scale which are similar to the size of important biomacromolecules such as proteins, and DNAs, which control the excretion from the body.
2. Huge number of surface groups usable for conjugation of targeting moieties, signaling groups, drugs, or biocompatibility groups. Their conjugation can influence biodistribution, receptor-mediated targeting, therapy dosage, or controlled release of drugs from the interior space. As well, this conjugation augments or resists trans-cellular, epithelial, or vascular biopermeability.
3. Non- or low-immunogenicity which can be achieved by conjugation of surface groups with PEG.
4. Small molecule drugs, metals, or probes can be encapsulated in the interior void space.
5. Desired biocompatibility is correlated with lower generation anionic or neutral polar terminal surface groups in contrast to higher generation neutral apolar and cationic surface groups.

These dendritic properties are the consequence of the structure, the uniformity of molecules, the collective and cooperative actions of internal and external functional groups, the large number of termini, the high local concentrations of these internal/external functional groups, the ability to behave as dendrimer pseudophase, and the soft to hard (organic) nanoparticle character (Svenson and Tomalia 2005; Tomalia 2005a; Svenson 2007; Balogh 2007; Chabre and Roy 2008; Newkome and Shreiner 2008; Niederhafner et al. 2008a). The number of terminal groups increases exponentially with increasing generations, and the overall density of the dendrimer molecules increases as well. In contrast, the flexibility of the dendrimer and accessibility of all functional groups decreases. Overall

density and rigidity increased by high level of symmetry lead to limited interpenetration between molecules. High density of terminal groups limits the number of their intermolecular interactions, and prevents the dendrimer penetration. The interactions occur on the surface of the dendrimer (Balogh 2007; Tomalia 2009).

The folding of the dendrimers is strongly solvent dependent. When the solvent is compatible with terminal groups, they are expanded, but not disentangled. On the other hand, incompatible solvent induces burial of functional groups inside the dendrimers (Balogh 2007; Svenson 2007, 2009; Svenson and Chauhan 2008).

The understanding of pH-controllable supramolecular systems including dendrimers led to the construction of important molecular machines for electronic and biological applications (Leung et al. 2009). They can be controlled by simple perturbation with acids and bases.

A biological function of proteins and peptides strongly depends on proteolysis (Sommer et al. 2009). Branched peptides (MAPs) are resistant toward proteolysis by trypsin and α-chymotrypsin. The protease reactivity of peptide dendrimers was controlled by the degree of branching. Dendrimers with two or more amino acids between branching points were easily cleaved by trypsin independently on the position of the reactive sequence within the dendrimers, whereas more compact dendrimers with only one amino acid between branching points were resistant toward trypsin cleavage. The topology controlled proteolysis provides a novel possibility for tuning of the biological properties of peptide dendrimers, which are not available in linear peptides.

Dendrimers are capable to form supramolecular structures via self-assembly (Rosen et al. 2009; Wang and Kaifer 2009). These supramolecules can serve for molecular recognition.

Since the mass of dendrimer increases exponentially and the volume increases cubically with the number of generations, a maximum is observed in the plot of log intrinsic viscosity versus molecular weight. Because intrinsic viscosity is inversely proportional to the solution density, the density should display a minimum when maximal intrinsic viscosity is observed. As well, in that point, minimal refractive index is expected (Balogh 2007; Boas et al. 2006g; Svenson 2009). During acido-basic titration of polyionic dendrimers (i.e. with nitrogen, or phosphorus as branching atom; with carboxylates as functional group, etc.), the separation of nanophases was observed. Due to strong electrostatic repulsions of identical charges (protonated or deprotonated functional groups of dendrimer), large structural reorganizations take place in order to minimize energy of the molecule. These reorganizations lead to collapses or expansions of dendrimers and consequently nanophase-separation occurs (Balogh 2007; Svenson 2009; Svenson and Tomalia 2005; Tomalia 2005a, 2009).

Dendritic chain reaction (DCR) (Sella and Shabat 2009) is a signal amplification technique that uses the disassembly properties of self-immolative dendrimers (Fig. 1). These dendrimers liberate the end-group molecules by domino-like reactions activated by a single event. Since compounds released from the end-groups acquire the chemical reactivity for activation of another dendritic molecule, a capture of single signal molecule leads to a chain reaction that disintegrates all of the dendritic molecules through an exponential progress, ultimately releasing each of the end groups. Advantage of the process with exponential amplification of signal is very highly sensitivity for appropriate analyte.

Detection of H_2O_2 was described by DCR technique using AB_3 dendron (Sella and Shabat 2009). The dendron consists of a 4-nitroaniline reporter, two choline units, and phenylboronic acid as a trigger. The boronic acid reacts with hydrogen peroxide under mild alkaline conditions to generate intermediate phenylborate, which is then hydrolyzed to produce the corresponding phenol. This phenol further decomposes via several eliminations to 4-nitroaniline and the two choline molecules, i.e. the cleavage generates one chromogenic reporter and two choline molecules. Oxidation of two free cholines to betaines with choline oxidase provides four molecules of hydrogen peroxide, which then activate additional four AB_3 dendrons. The rate of the reaction should increase exponentially until each of the 4-nitroaniline molecules has been liberated.

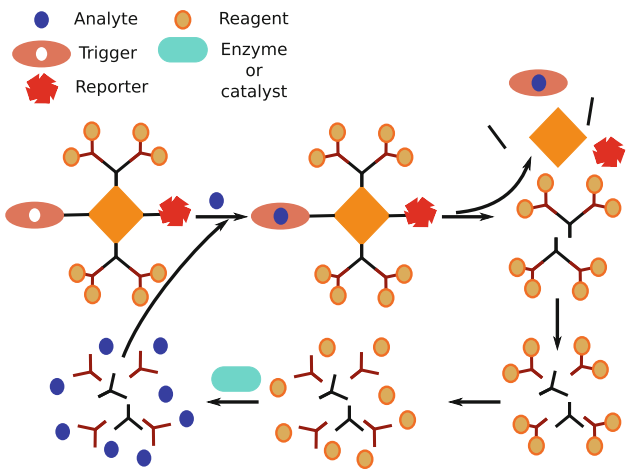

Fig. 1 Schematic illustration of dendritic chain reaction (DCR) (Sella and Shabat 2009). When the blue analyte is captured by the trigger, the spontaneous decomposition of self-immolative dendrimer is initiated. During the decomposition, the reporter molecule, which can be detected via UV–VIS or fluorescence spectroscopy, is released. The reagent is another product of the decomposition, which is converted by the enzyme or the catalyst to the analyte. This is the key step of the signal amplification

The yellow color of the released 4-nitroaniline can be evaluated. Due to the amplification of background signal of spontaneous hydrolysis, the sensitivity of this system allowed detection of analyte down to only 5 μM H_2O_2. Nearby the limit of the assay sensitivity, the signal of the DCR technique was 53-fold stronger than that obtained without amplification. Because the DCR-based assay can be coupled with another probe, detection of other analytes and biocatalysts is possible.

Structure of dendrimers in the solid phase is significantly different than that in solution because removal of solvent molecules leads to collapse of the spheroidal shape (Balogh 2007; Svenson 2009; Svenson and Tomalia 2005; Tomalia 2005a, 2009). Polymer-like glass transition temperatures (T_g) of these materials are observed during the first run of differential scanning calorimetry. This phenomenon is not observed on the consecutive runs. It requires extremely long annealing time until the materials solidify and recover. Generally, dendrimers are amorphous and do not form crystals, because of their symmetry and spherical shape. Their T_g is not usually high and depends on their family and generation. Their mechanical properties can be improved by their conjugation into cross-linked polymeric systems. Dendrimers in molten state form ideal Newtonian liquids (Balogh 2007; Svenson 2009; Svenson and Tomalia 2005; Tomalia 2005a, 2009).

To reduce a dependency of the world on fossil-fuel energy, the idea of replacing petroleum-based plastics with hydrogels emerged. Preparation of a transparent hydrogel from water, clay (2–3% by mass) and a very small proportion (<0.4% by mass) of organic components was described (Wang et al. 2010a). The hydrogels can be molded into shape-persistent and free-standing objects. They rapidly and completely self-heal when damaged.

Two models are used for explanation of the chirality amplification in dendrimeric polymers by cooperative conformational equilibria: the "sergeants and soldiers" principle and the "majority rules" model (Lockman et al. 2005). The presence of small proportions of chiral monomers within a starting building block composed of predominantly achiral monomers results in the predominance of a single helical sense. Amplification of the small energetic bias by the rarity of helical reversals is responsible for forcing the helix to adopt a uniform helical sense (Lockman et al. 2005). In the above "kickline" analogy, a chiral monomer which kicks in a fixed direction drives its neighbors to "kick" in the same direction to prevent destabilization due to mismatched kicking. Unwinding of formed helix is energetically disfavored, and thus this directionality propagates down the polymer and results in a large chiral amplification. Since a small number of chiral "sergeants" directs the conformational properties of a large number of achiral "soldiers", this is termed as the "sergeants and soldiers" effect. Polymerization of a mixture of enantiomeric monomers with small enantiomeric excess leads to dendritic polymer, which exhibits a helical bias identical to the corresponding chiral homopolymer. Since this effect leads to chirality amplification of the major enantiomer, it is called the "majority rules" principle (Lockman et al. 2005).

The propagation of a falling motion of one dancer throughout the line shows the effect of a localized perturbation on the conformation of macromolecular system. Further amplification is achieved by aggregation, which influences the helical reversals during the helix association. It leads to the shifts of helical equilibrium toward segments with higher helical bias due to their association in solution (Lockman et al. 2005).

A systematic investigation of every layer of dendrimers by incorporating a single ferrocene unit in well-defined locations in dendrons was carried out (Azagarsamy et al. 2009b). The redox potential values of ferrocene at intermediate layers were remarkably different from those at the core and the periphery. In spite of location-dependency of redox potential values, no significant change in the rate of heterogeneous electron transfer (k_0) was observed with respect to locations. This was explained by the possibility to nullify the distance between the electrode and ferrocene unit by free rotation of dendrimer.

Dendrimeric effects

Glycocluster

Glycocluster is a sterical arrangement of two or more glycotopes, e.g. in the form of dendron or dendrimer, leading to the amplification of the given biological or physicochemical activity. The amplification factor is a few orders of magnitude higher than the sum of the individual contributions. For more details see "Cluster effect" (Chabre and Roy 2010; Roy 2003; Lindhorst 2002; Roy and Baek 2002; Roy and Kim 2003; Kohn et al. 2004; Singh et al. 2005; Kalovidouris et al. 2003; Andre et al. 2004b; Patel and Lindhorst 2006; Aoyama 2004; Nakai et al. 2003; Aoyama et al. 2003; Lundquist and Toone 2002; Gao et al. 2004; Grandjean et al. 2002; Sato et al. 2003, 2006; Oshovsky et al. 2004; Hada et al. 2005; Westermann and Dorner 2005; Hayashida et al. 2003; Touaibia and Roy 2007; Zhang et al. 2009; Branderhorst et al. 2008; Chabre and Roy 2008; Imberty et al. 2008; Roy and Touaibia 2007; Niederhafner et al. 2008a).

Multivalency

Multivalency is a prerequisite to attain biologically useful affinities between carbohydrate ligands and their protein

receptors (Lindhorst 2002; Turnbull and Stoddard 2002; Ouerfelli et al. 2005; Pieters 2007; Rosa Borges and Schengrund 2005; Smith 2006; Wolfenden and Cloninger 2005; Benito et al. 2004; Wu et al. 2004; Kitov and Bundle 2003; Sung et al. 2006; Boas and Heegaard 2004; Heegaard and Boas 2006; Chabre and Roy 2010). Arrangement of the carbohydrate epitopes as multiple copies on an appropriate scaffold (polymeric, molecular, and dendritic) creates a multivalent display that can effectively mimic the nature of affinity enhancement. As a result, higher affinities than expected from the sum of the individual interactions are obtained. This concept was termed "cluster effect". Cooperativity and multivalency in dendrimer and supramolecular chemistry have been reviewed (Badjic et al. 2005; Gabius 2008; Chabre and Roy 2008). Synthetic tailor-made multivalent architectures, provided with non-covalent bonding interactions as the supramolecular "glue", represent (i) well-defined systems for studying the concept of multivalency in nature and (ii) building blocks for nanomaterials.

"Multivalency has demonstrated that precise design and strong individual interactions are not necessary if recognition of a protein surface should be achieved. The sum of weaker contacts in a flexible context around a central scaffold can be much more efficient and even selective" (Martos et al. 2008).

Cluster effect

In general, interactions between saccharides and peptides or proteins are weak. Isolated carbohydrate–protein interactions are typically very weak with K_D values in the range of 10^{-3}–10^{-6} M. The nature compensates for the weakness of these isolated interactions by tending to cluster together multiple copies of carbohydrate ligands and their receptors. In this way, stronger cooperative binding takes place, known as "cluster effect" or "multivalent effect" (Chabre and Roy 2010; Roy 2003; Roy et al. 1993; Lundquist and Toone 2002; Tsvetkov and Nifantiev 2005; Boas and Heegaard 2004; Wolfenden and Cloninger 2005; Matsuura and Kobayashi 2004; Roy and Touaibia 2007; Turnbull and Stoddard 2002; Niederhafner et al. 2008a; Gabius 2008; Zhang et al. 2009).

Roy et al. (1993) prepared sialosyl MAGs with valencies of 2, 4, 8, and 16. To the lysine branches, sialic acid was bound by S-CH_2-CO-Gly-Gly spacer. Binding properties of the sialylated MAGs were studied using the plant lectin wheat germ agglutinin (WGA) in a direct enzyme-linked lectin assay (ELLA) in microtiter plates using horseradish peroxidase (HRPO) labeled WGA. Best binding properties were obtained with the octa- and hexadecavalent MAG. In an inhibition test using sialylated glycopolymer as coating antigen and HRPOWGA, all MAGs showed excellent inhibitory capacities (106 times better than a monosialoside). The hexadecavalent MAG was the most powerful inhibitor.

The effect of multivalency concept (cluster effect) has been clearly proven (Turnbull and Stoddard 2002; Bezouska 2002; Pieters 2004, 2006; Branderhorst et al. 2008; Roy et al. 2001; Renaudet and Dumy 2003; Heegaard and Boas 2006; Arosio et al. 2005; Wu et al. 2004; Sung et al. 2006; Matsuura and Kobayashi 2004; Page et al. 1996; Grandjean et al. 2001; Lee et al. 2005b; Imberty et al. 2008). The cluster glycoside effect was studied also by calorimetric analysis of multivalent glycodendrimer ligands (Corbell et al. 2000; Dam and Brewer 2004). Multivalent glycosides (glycodendrimers, glycopeptide dendrimers) bind to their polyvalent protein receptors with affinities much greater than those that can be explained solely on the basis of valency. This effect was studied on mannosylated dendritic ligands and their performance in competitive and non-competitive binding assays, e.g. hemagglutination inhibition, ELLA, and isothermal titration microcalorimetry (ITC). Consistency between the thermodynamic parameters of association and non-specific aggregation, rather than enhanced lectin–ligand affinity was found.

Interpretation of cluster glycoside effect on molecular level has been reviewed (Pieters 2004; Lundquist and Toone 2002; Kitov and Bundle 2003; Dam and Brewer 2004). The authors consider three mechanisms for the cluster effects: intramolecular, intermolecular, and steric stabilization. In general, we can say with some exaggeration (Niederhafner et al. 2008a) that in the case of cluster effect 1×8 is a few orders of magnitude higher than 8×1. The optimal results depend on the structure of the dendrimer, its surface groups, methodology used, model (in vivo, in vitro), and many others. In some cases, for the given activity, valency 4 is better than 16, in other cases it can be the opposite (steric reasons) (Roy and Baek 2002). In most cases, tetravalent dendrimers give the best results. The optimal valency for any dendrimer and its application must be determined separately. In any case, some optimization is necessary for successful biological output. Such optimization led to compound with 440-fold enhancement in potency over L-fucose, i.e. 55-fold enhancement over-calculated to one L-Fuc in the dendrimer (Kolomiets et al. 2009; see also "Dendrimeric libraries").

Besides "cluster effect" or "multivalent effect", other terms like multivalent glycotope (Singh et al. 2006) and clustering effect (Bezouska 2002) have also been coined.

Macromolecular effect

Not all interactions can be explained by multivalency or cluster effect. Lindhorst proposed that understanding of

fimbriae-mediated bacterial adhesion might require at least two different points of view (Dubber et al. 2006b). One approach deals with results obtained from hemagglutination inhibition assays or ELISA. They observed an inhibition of bacterial adhesion that can be neither rationalized on the basis of the known crystal structure of FimH nor interpreted in the sense of a classical "multivalency effect". The inhibition of bacterial adhesion to the glycocalyx or a glycocalyx mimetic should be rather explained by a "macromolecular effect" (Dubber et al. 2006b). The inhibitory potencies can be correlated to features which are typical for macromolecules and the interactions they form, rather than for distinct molecular epitopes (Dubber et al. 2006b). The macromolecular effect was discussed also by Morvan et al. (2007).

Synthesis of dendrimers: convergent and divergent approaches

Dendrimer synthesis can be in general performed by two major strategies (Fig. 2) (Svenson and Tomalia 2005; Tomalia 2005a; Roy and Touaibia 2007; Rosen et al. 2009; Nanjwade et al. 2009). The first one is "the divergent method" in which growth of a dendron originates from a core site. In the divergent method, monomeric modules are assembled in a radial, branch-upon-branch motif according to certain dendritic rules and principles. The second method is based on a "convergent synthesis". It starts from what will change into the dendrimer shell inward to a reactive focal point, creating a single reactive dendron. A dendrimer structure is formed by reaction of several dendrons with a multifunctional core. The significant improvements of both convergent and divergent strategies

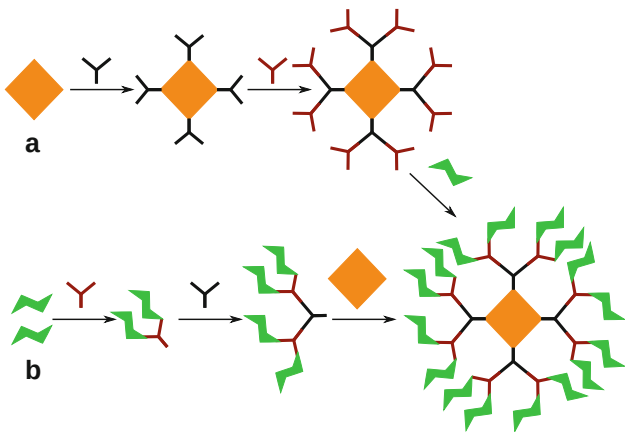

Fig. 2 Divergent (**a**) and convergent (**b**) approaches of dendrimer synthesis (Roy 2003; Svenson and Tomalia 2005; Tomalia 2005a; Roy and Touaibia 2007; Rosen et al. 2009; Nanjwade et al. 2009; Chabre and Roy 2008)

of dendrimer synthesis are "Lego", "click", and ligation chemistries. They are described in individual sections.

Divergent approach was used as a guidance rule in dendrimer synthesis because of its major advantages, such as cheap reagents, fast synthesis, exponential growth, and possibility to prepare large dendrimers (Rosen et al. 2009; Li et al. 2007; Nanjwade et al. 2009; Chabre and Roy 2008; Roy and Touaibia 2007; Dufes et al. 2005; Villalonga-Barber et al. 2008; Carlmark et al. 2009; Boas et al. 2006a, g; Roy 2003; Tomalia 2005a; Balogh 2007; Kehat et al. 2007; Cheng et al. 2008b; Niederhafner et al. 2005; Boas and Heegaard 2004; Svenson 2007). However, purification of compounds prepared by divergent strategies is more complicated, due to the contamination of the desired product by thousands of deletion compounds with molecular weight, charge, polarity, hydrophilicity, etc. very similar to the desired product. Moreover, the steric resistance of higher generation dendrimers can prevent couplings of next building blocks and can lead to significant defects on the surface of dendrimer. Thus, it is necessary to purify the growing glycodendrimer in every generation in order to suppress cumulative effects which stem from failed couplings (Rosen et al. 2009; Li et al. 2007; Chabre and Roy 2008; Roy and Touaibia 2007; Dufes et al. 2005; Villalonga-Barber et al. 2008; Carlmark et al. 2009; Boas et al. 2006a, g; Roy 2003; Tomalia 2005a; Balogh 2007; Kehat et al. 2007; Cheng et al. 2008b; Niederhafner et al. 2005; Boas and Heegaard 2004; Svenson 2007).

In summary, the divergent approach is an easy way for preparation of dendrimers, either by chemical ligation or connection of carbohydrates and dendrimers with linking bridges.

In contrast to the divergent approach, the convergent one has advantages, such as monodisperse dendrimer, the capability of attaching different types of dendrons to one dendrimer, and easier purification and characterization of the product (Rosen et al. 2009; Nanjwade et al. 2009; Li et al. 2007; Chabre and Roy 2008; Roy and Touaibia 2007; Dufes et al. 2005; Ozawa et al. 2007; Villalonga-Barber et al. 2008; Carlmark et al. 2009; Boas et al. 2006a, g; Roy 2003; Tomalia 2005a; Hackenberger and Schwarzer 2008; Balogh 2007; Kehat et al. 2007; Cheng et al. 2008b; Niederhafner et al. 2005; Boas and Heegaard 2004; Svenson 2007). The purification is easier because the byproducts differ from the desired structure in that the whole "arm" may be missing, and therefore the molecular weight, charge, etc. are different enough to achieve successful separation by some of the techniques mentioned below. During the trials of attaching large dendrons to the central core, the synthesis could be limited by steric constraints. Both approaches have advantages and disadvantages, and the appropriate strategy of dendrimer synthesis depends mainly on the designed structure and generation (Li et al. 2007; Chabre and Roy 2008; Roy and Touaibia

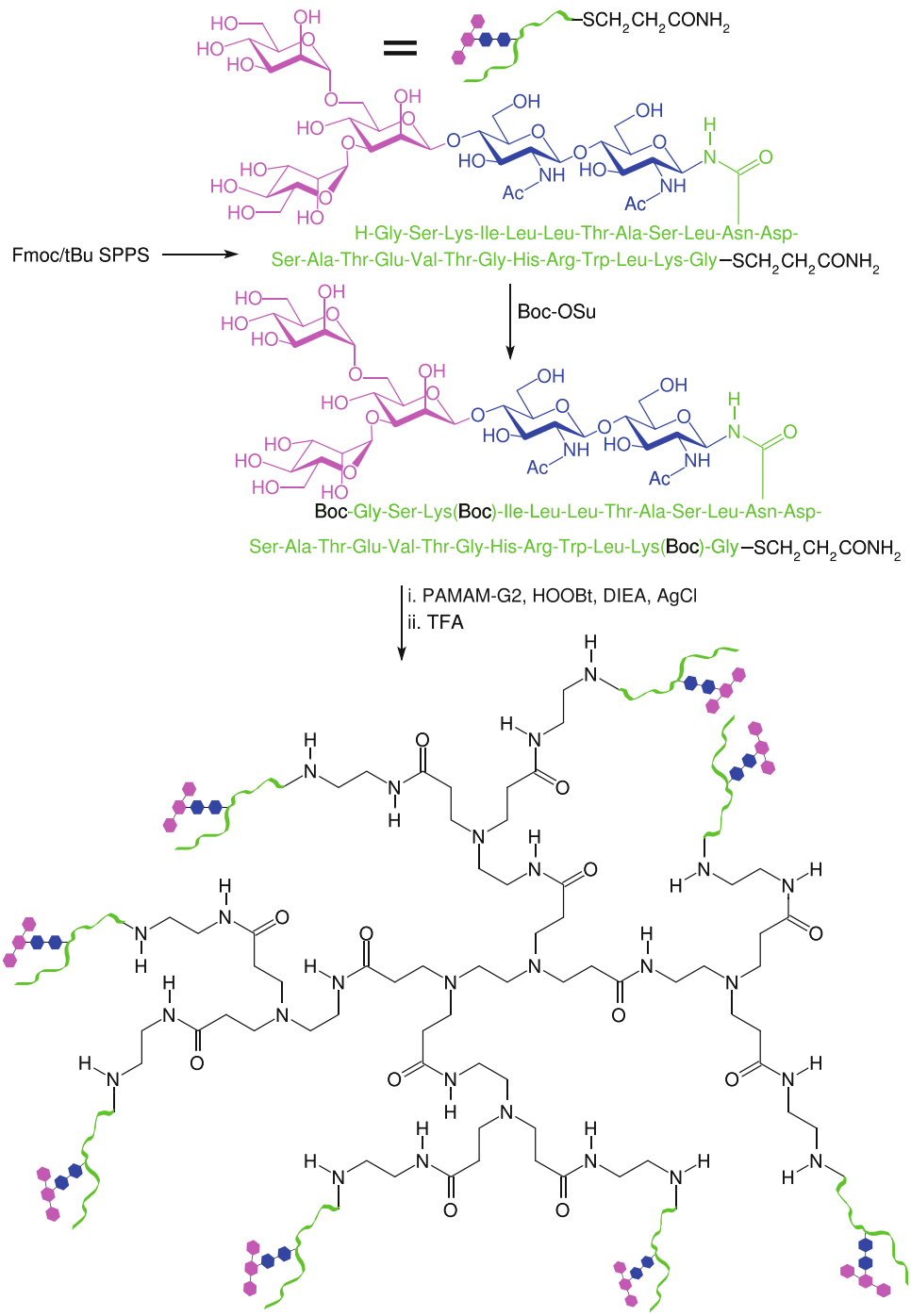

Fig. 3 Convergent synthesis of glycopeptide dendrimer via chemical ligation (Ozawa et al. 2007)

2007; Dufes et al. 2005; Carlmark et al. 2009; Boas et al. 2006a, g; Roy 2003; Tomalia 2005a; Hackenberger and Schwarzer 2008; Balogh 2007; Kehat et al. 2007; Cheng et al. 2008b; Boas and Heegaard 2004).

In general, many carbohydrates, spacers, and repeated units can serve as building blocks for convergent construction of dendritic structures providing a broad platform for the design of glycodendrimers with versatile properties. These examples suggested that the development of glycodendrimers for biological applications by the convergent approach was also promising. In this way it is possible to overcome the relatively low sugar loading efficacy in the divergent approach caused by steric hindrance (Li et al. 2007).

There are several examples of dendrimer syntheses. Synthesis of glycopeptide dendrimer by a convergent method has been described (Fig. 3). Glycopeptide thioester R-Gly-Ser-Lys(R)-Ile-Leu-Leu-Thr-Ala-Ser-Leu-Asn($Man_3GlcNAc_2$)-Asp-Ser-Ala-Thr-Glu-Val-Thr-Gly-His-Arg-Trp-Leu-Lys(R)-Gly-$SCH_2CH_2CONH_2$ (R = Boc) comprising

the sequence of extracellular matrix metalloproteinase inducer (emmprin) (34–58) was prepared by Ozawa et al. (2007) by SPGS and condensed with an octavalent PAMAM dendrimer core having eight amino groups by the thioester method (2 equiv. to each hand of the dendrimer core). After condensation, the Boc groups were removed by TFA. The desired product, a glycopeptide dendrimer carrying an *N*-linked core pentasaccharide of about 30 kDa, was successfully isolated and characterized by MALDI-TOF analysis. The desired product was obtained in a low yield. Attempts to remove side products by reverse-phase HPLC failed. The authors found that SDS-PAGE retained sufficient resolution to remove these impurities. The results of the MALDI-TOF analysis indicate the successful purification of the desired glycodendrimer. Amino acid analysis of the product also supported the success of the synthesis. The quantitative introduction of eight glycopeptide chains to the dendrimer core is difficult. Thus, a novel method has to be developed to achieve a more efficient preparation of larger glycopeptide dendrimers.

Divalent and tetravalent polyether glycodendrons have been prepared by convergent method. However, the synthesis of higher generation polyether glycodendrons was not possible. The polyether glycodendron chemistry used for incorporation of galactose moieties and to the synthesis of glycodendrons of a "mixed" type was extended (Elsner et al. 2007), involving the scaffolding of both mannosyl and galactosyl residues. Derivatization of the focal points of these compounds is necessary in order to use these glycomimetics for biological applications.

The syntheses of monodisperse lysine MAGs (Greatrex et al. 2009), G0 to G5 with benzhydrylamine at the core, with 2 to 64 mono-, di-, and tri-α-mannopyranosyl residues were described by divergent approach. Reactive *N*-hydroxysuccinimide esters were used to ensure complete reaction of dendrimer amines with the mannosylating reagents. The purity of the MAGs was checked by RP-HPLC and MALDI-TOF MS and was found to be excellent. MALDI-TOF measurements were more useful than NMR for visualizing impurities, especially on higher generation materials. The relative ability of these MAGs to induce dendritic cell maturation was measured, however, no significant trends were observed.

Fluorophore-labeled MAGs were prepared by divergent SPGS. The authors (Kantchev et al. 2008) elaborated a direct, expedient MAG synthesis from commercially available or easily prepared building blocks by automated Fmoc/Bu^t-SPGS. This method allows a large excess of reagents to be used in order to drive the reaction to completion, thereby minimizing imperfections within the dendritic structure. Large, monodisperse G4 and G5 MAGs were prepared and capped with 16 and 32 mannose residues, respectively, in a single synthetic operation, yielding [Man-α-O-$(CH_2)_3$CO]$_{16}$-$K_8K_4K_2$KF-$CONH_2$ (G4 glycodendron), and [Man-α-O-$(CH_2)_3$CO]$_{32}$-$K_{16}K_8K_4K_2$KF-$CONH_2$ (G5 glycodendron). Incorporation of a C-terminal lysine residue in the G4 MAG allows fluorescence labeling with a number of common labels on resin, in organic solvent or in aqueous buffer, as required. The labeled MAGs [Man-α-O-$(CH_2)_3$CO]$_{16}$-$K_8K_4K_2$KK(FITC)-$CONH_2$, [Man-α-O-$(CH_2)_3$CO]$_{16}$-$K_8K_4K_2$KK(pyrene)-$CONH_2$, [Man-α-O-$(CH_2)_3$CO]$_{16}$-$K_8K_4K_2$K(Dns)-$CONH_2$, and [Man-α-O-$(CH_2)_3$CO]$_{16}$-$K_8K_4K_2$KK(Rho)-$CONH_2$ were used to study carbohydrate–protein interactions by fluorescence spectroscopy and imaging methods. A single HPLC purification was sufficient in all cases to obtain a homogeneous sample. The purity of the MAGs was confirmed by MALDI-TOF. The FITC-labeled G4 MAG is an excellent probe for the imaging studies of mannose-receptor-mediated entry into dendritic cells by confocal fluorescence microscopy.

Lego chemistry

Two series of publications with intriguing terms "Molecular Meccano" (Anelli et al. 1992; Amabilino et al. 1995) and "Molecular Lego" have been published. The term "Molecular Lego" was first used by Stoddart (Stoddart 1988; Ellwood et al. 1988; Kohnke et al. 1989) in 1988. Both are the trade names of child's toys in which complicated structures are built from a limited number of simpler bricks.

Lego chemistry was used for the straightforward synthesis of dendrimers (Maraval et al. 2003; Svenson and Tomalia 2005; Villalonga-Barber et al. 2008; Svenson 2007) and also in general organic chemistry (Meldal et al. 2010; Mano and Kuhn 2005; Schafmeister 2007).

Molecular Lego represents a modest collection of small building blocks which enable the design and synthesis of nanoscale structures programmed to have virtually any shape desired. (Meldal et al. 2010; Schafmeister 2007).

It is based on attaching simple modules to each other in a facile manner (Mathias and Stoddart 1992) and was used for the preparation of macromolecules (Meldal et al. 2010; Borner 2007). One limitation to Lego strategy is that, while it is very powerful method for the rapid development of dendritic structures, the choice of monomers is partly restricted since care must be taken to ensure that the reactions involved in dendrimer growth do indeed proceed with the required selectivity (Villalonga-Barber et al. 2008).

Ligations

Special cases of convergent approaches are chemical ligations. They are based on chemoselective reactions, which are suitable for coupling of non-protected peptides,

glycopeptides, and other substances, among them, condensation of amines, hydroxylamines, and hydrazines with aldehydes; reactions of aldehydes with vicinal amino alcohols and amino thiols; coupling of thioacids with alkylhalides; thioesters with vicinal aminothiols; phosphines with azides; and several reactions belonging to click chemistry, etc. (see "Click chemistry"). These types of reactions have been widely reviewed (Payne and Wong 2010; Sletten and Bertozzi 2009; Hackenberger and Schwarzer 2008; Agard and Bertozzi 2009; van Dijk et al. 2009; Carrico 2008; Du et al. 2009; Niederhafner et al. 2005, 2008a; Kurpiers and Mootz 2009; Canalle et al. 2010; Tiefenbrunn and Dawson 2010; Toth et al. 2008; Lutz and Zarafshani 2008; Durek and Becker 2005; Nilsson et al. 2005; Brik and Wong 2007; Villalonga-Barber et al. 2008; Tron et al. 2008; Dondoni 2007; Gauthier and Klok 2008; Renaudet 2008; Roy and Touaibia 2007; Roy 2003; Laughlin and Bertozzi 2009).

Pseudoprolines were formed from peptide aldehydes and N-terminal Cys, Ser, and Thr using imine ligation by Tam's team (Liu and Tam 1994a, b; Tam and Miao 1999; Tam et al. 1999; Miao and Tam 2000; Eom et al. 2003). A bidirectional ligation mode for coupling of three unprotected segments in tandem to form two pseudoproline bonds (thia- or oxaproline) was described (Miao and Tam 2000). The ligation in the $C \rightarrow N$ direction is allowed by the chemoselectivity of an amino terminal Cys over a Ser or Thr peptide with a peptide that has a carboxyl terminal glycolaldehyde ester. For ligation in opposite direction, the masking of aldehyde function as glycerol ester was employed. This tandem process can be extended for ligation of three or more segments. The pseudoproline formation was further studied by others (Tuchscherer and Mutter 2005; Wathier et al. 2006).

The peptide aldehydes also undergo chemoselective reactions with hydrazines and *N*-oxyamines, which lead to corresponding hydrazides and oximes in reversible manner (Gaertner et al. 1992; Rose 1994; Flavell et al. 2008; Ye et al. 2008; Kalia and Raines 2008; Dirksen and Dawson 2008; Canalle et al. 2010; Tiefenbrunn and Dawson 2010; Hoiberg-Nielsen et al. 2008; Tofteng et al. 2007; Boturyn et al. 2008). Since the hydrazides and oximes are good nucleophiles with lower pK_a values than that of amines, they can react with aldehydes under slightly acidic condition, where the basic side chains of amines are protected from the reaction by protonation. In order to stabilize these compounds, borohydride reductions of corresponding hydrazides and oximes were carried out (Canalle et al. 2010; Tiefenbrunn and Dawson 2010). The formation of hydrazides and oximes reaches its equilibrium fast, when aniline is used as catalyst (Dirksen et al. 2006a, b; Dirksen and Dawson 2008; Kohler 2009; Lempens et al. 2009). This fact could be used in formation of dynamic combinatorial libraries (DCLs). These reactions were carried out from peptide precursors and should be useful for various protein derivatization and ligation strategies in the near future. For other examples see Sletten and Bertozzi (2009), Hackenberger and Schwarzer 2008, Agard and Bertozzi (2009, Carrico (2008), Kohler (2009), Lempens et al. (2009), Canalle et al. (2010), and Tiefenbrunn and Dawson (2010).

To achieve higher stability of ligation products and to avoid unnatural linkage, ligation strategy leading to an amide bond was developed. Kemp studied 4-hydroxy-6-sulfanyldibenzofuran auxiliary for ligation of protected peptides (Kemp and Galakatos 1986; Kemp et al. 1986; Fotouhi et al. 1989; Kemp and Carey 1993). The first step is the sulfanyl-capture reaction, leading to the formation of a disulfide bridge between the sulfanyl group of the N-terminal cysteine present in one segment and the sulfanyl functionality of the template attached at the C-terminus of the second segment. The second step is the acyl transfer reaction which is responsible for the formation of the amide bond between the two fragments. First, Kemp's group investigated the ligation of protected peptides (Kemp and Galakatos 1986; Kemp et al. 1986), later, after Kent's publication of chemical ligation of unprotected segments in aqueous solution (Schnolzer and Kent 1992), Kemp also showed that "prior-capture ligation" can be carried out with unprotected segments in aqueous environment (Kemp and Carey 1993). Moreover, Kemp's "prior-capture ligation" forms natural amide bonds, whereas the Kent's first method (Schnolzer and Kent 1992) led to formation of a thioester related with the desired protein.

In 1994, Kent published the second method (Dawson et al. 1994) called "native chemical ligation". Like his previous method (Schnolzer and Kent 1992), which allows synthesis of the all-D-protein (Milton et al. 1992), it uses an enhanced nucleophilicity of sulfur. In the previous method, the chemoselective reaction of alkyl bromide with sulfanyl group was used. The disadvantage was formation of protein containing thioester bond, which is not stable toward hydrolysis under physiological conditions. In the new method, the ligation of C-terminal thioester with N-terminal Cys was described. It led to an amide bond between the AA–Cys and thus the native protein was obtained. This opened a facile way for syntheses of several important proteins and glycoproteins (Payne and Wong 2010; Shekhter et al. 2010; Skrisovska et al. 2010; Hirano et al. 2009; Garner et al. 2009; Kana and Danishefsky 2009; Muir et al. 1998; McGinty et al. 2009; Flavell and Muir 2009; Bayley et al. 2009; Komarov et al. 2009; Li et al. 2009; Torbeev and Kent 2007; Hojo et al. 2005). The building blocks for the native chemical ligation can be obtained by both chemical (Blanco-Canosa and Dawson 2008; Mende and Seitz 2007; Flemer 2009; Nakamura et al. 2009; Bang et al. 2006a; Johnson and Kent 2007;

Schnolzer et al. 2007; Hojo et al. 2005, 2007, 2008; Brunsveld et al. 2006; Wang and Miranda 2005; Gross et al. 2005; Ollivier et al. 2005; Brask et al. 2003a; von Eggelkraut-Gottanka et al. 2003; Quaderer and Hilvert 2001; Swinnen and Hilvert 2000; Sewing and Hilvert 2001; Li et al. 1998; Zhang and Tam 1997; Kimmerlin and Seebach 2005; Hackenberger and Schwarzer 2008) and recombinant approaches. The later ones use intein-based techniques for synthesis of protein thioester segment and specific proteases for synthesis of segment containing N-terminal Cys. This expressed protein ligation has been deeply reviewed (Muir et al. 1998; McGinty et al. 2009; Flavell and Muir 2009; Skrisovska et al. 2010; Bayley et al. 2009; Komarov et al. 2009; Payne and Wong 2010; Li et al. 2009; Mootz 2009; Berrade and Camarero 2009; Flavell et al. 2008; David et al. 2004). To achieve full convergence of the native chemical ligation, repeated ligations were studied by both modular approach using N-terminal Cys protection (Bang and Kent 2004; Bang et al. 2005) and approach based on kinetically controlled reaction conditions (Bang et al. 2006b; Rajagopal and Kent 2007; Durek et al. 2007; Blanco-Canosa and Dawson 2008).

A limitation of the native chemical ligation is a low abundance of naturally occurring Cys residues (Miseta and Csutora 2000). Several approaches were described to address this issue. They were based on (1) an introduction of a cleavable/permanent auxiliary group on the α-amino group of N-terminal amino acid in the junction (Payne and Wong 2010; Kana and Danishefsky 2009; Hackenberger and Schwarzer 2008; Haase and Seitz 2008; Kimmerlin and Seebach 2005); (2) an introduction of an auxiliary sulfanyl group to amino acid side chain, which can be removed by reduction or alkylated (Garner et al. 2009; Haase et al. 2008; Wan and Danishefsky 2007; Crich and Banerjee 2007; Pentelute and Kent 2007; Pasunooti et al. 2009; Rajagopal and Kent 2007; Kana and Danishefsky 2009; Payne and Wong 2010; Hackenberger and Schwarzer 2008; Yang et al. 2009b; Haase and Seitz 2008); (3) an introduction of an auxiliary sulfanyl group on a sugar moiety (Payne and Wong 2010; Hackenberger and Schwarzer 2008; Bennett et al. 2008; Brik and Wong 2007); and (4) using partial protection of amino groups (Chen et al. 2007; Katayama et al. 2008). Native chemical ligation–desulfurization strategy was applied for synthesis of homogeneous glycopeptide analogs of fish antifreeze glycoproteins of discrete oligomeric length (Garner et al. 2009).

Efficient and systematic synthesis of a small glycoconjugate library containing human complex type oligosaccharides was achieved by native chemical ligation (Murase et al. 2009).

Sugar-assisted ligation (SAL) is a useful method for the convergent construction of complex glycopeptides (Bennett et al. 2008; Payne and Wong 2010; Hackenberger and Schwarzer 2008). SAL with the thiol auxiliary was elaborated for syntheses of glycopeptides and glycoproteins. The effects of glycosylation at C-3, C-4, and C-6 of the C-2 auxiliary-containing glycan were studied. The study revealed that SAL is sensitive to extended glycosylation on the auxiliary-containing sugar. It is possible to carry out SAL with extended glycosylation at C-4 and C-6. However, C-3 glycosylation completely prevented the ligation reaction. SAL was facilitated by a substrate (Payne et al. 2007), when the glycopeptides contain up to six amino acid extensions at the N-terminus of the glycosylated residue.

Ligations of glycopeptide aryl esters of *o*-ethyldisulfanylphenol with/without *N*-cysteine glycopeptide were broadly employed by Danishefsky's group (Payne and Wong 2010; Hackenberger and Schwarzer 2008; Kana and Danishefsky 2009).

An erythropoietin (EPO) analog concentration above 50 pg mL^{-1} caused cell proliferation (Hirano et al. 2009). The EPO analog with two human complex-type sialyloligosaccharides was synthesized by combined use of chemical synthesis and protein expression in *E. coli*. Positions 24 and 30 are glycosylated, but the two sialyloligosaccharides do not negatively influence the binding of the EPO analog to a receptor.

Native chemical ligation was used for synthesis of anti-inflammatory peptide-functionalized hydrogels capable of encapsulation of insulin-secreting cells (Su et al. 2010). The network maintained the viability of encapsulated islet cells in the presence of a combination of cytokines including TNF-α, IL-1β, and INF-γ.

Japanese authors (Ozawa et al. 2008) designed a new method for synthesis of a highly pure glycopeptide dendrimer using the potential of the post-SPPS thioesterification, in which *N*-alkyl cysteine (NAC) at the C-terminus of the peptide was used as an *N*- to *S*-acyl migratory device (Fig. 4). The advantages of the method were demonstrated by the synthesis of a glycopeptide dendrimer, which consisted of eight peptide chains of the tandem repeat region of MUC1 containing two T-antigens. The synthesis afforded a highly pure MUC1-derived octavalent glycopeptide dendrimer {[Ac-Val-Thr(T-antigen)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr(T-antigen)-Ala-Pro-Pro-Ala-His-Gly]$_4$-Lys$_2$-Lys-Gly-NH-CH$_2$-}$_2$ of 22 kDa using a sequential segment coupling, achieved by an NAC-assisted thioesterification. The key step was conversion of NAC amide bond to thioester one by shifting of an equilibrium using 3-mercaptopropionic acid.

Native chemical ligation was applied for design and synthesis of lipopeptide–carbohydrate assembled multivalent vaccine candidates (Zhong et al. 2009).

There are several other ligation techniques, such as His ligation (Hackenberger and Schwarzer 2008),

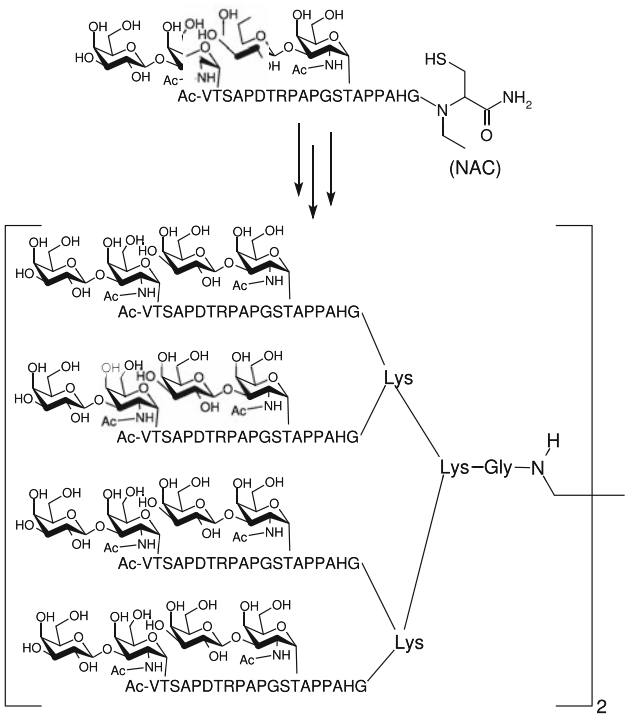

Fig. 4 *N*-alkyl cysteine (NAC)-assisted chemical ligation (Ozawa et al. 2008)

Staudinger ligation (Canalle et al. 2010; Hackenberger and Schwarzer 2008), sulfo-click ligation (Rijkers et al. 2010), enzyme catalyzed ligation (Kishan and Sharma 2010; Hackenberger and Schwarzer 2008; Payne and Wong 2010; Matsushita et al. 2009; Pritz 2008; Bennett and Wong 2007), etc.

Click chemistry

One of the most promising approaches for dendrimer synthesis is click chemistry. In his landmark review in 2001, Sharpless defined click chemistry as a group of reactions, which are modular, wide in scope, providing very high yields, form solely inoffensive byproducts that can be separated by non-chromatographic methods, and are stereospecific (but not necessarily enantioselective). The process should be simple (ideally, insensitive to oxygen and water), with readily available starting materials and reagents, it should require no solvent or a solvent that is easy to remove, and simple product isolation. If purification is required, it must be by non-chromatographic methods, and the product must be stable under physiological conditions (Kolb et al. 2001). Click chemistry (Meldal et al. 2010; van Dijk et al. 2009; Sletten and Bertozzi 2009; Hein et al. 2008; Chabre and Roy 2008; Niederhafner et al. 2008b; Roy and Touaibia 2007; Laughlin and Bertozzi 2009; Nandivada et al. 2007; Carlmark et al. 2009; Villalonga-Barber et al. 2008; Li et al. 2007; Lutz and Zarafshani 2008; Larsen et al. 2006; Kolb et al. 2001; Tron et al. 2008; Binder and Sachsenhofer 2007; Dondoni 2007; Imberty et al. 2008; Gauthier and Klok 2008; Touaibia and Roy 2007; Svenson 2007; Svenson and Tomalia 2005; Le Chevalier et al. 2009; Pourceau et al. 2008; Zhang et al. 2009; Agard and Bertozzi 2009; Wang et al. 2007; Hong et al. 2009) represents a group of reactions that are fast, simple to use, versatile, easy to purify, and regiospecific. Because of their high selectivity, near-perfect reliability, high yields, and exceptional tolerance toward a wide range of functional groups and reaction conditions click reactions have recently attracted increased attention, specifically for use in dendrimer and polymer synthesis, pharmaceutical sciences, construction of therapeutics, bioconjugates, biomaterials as well as for the modification of surfaces and nanometer and mesoscale structures. The topic of "clicked" glycodendrimers was reviewed with many examples and applications (Roy and Touaibia 2007; Dondoni 2007; Imberty et al. 2008; Touaibia and Roy 2007; Chabre and Roy 2008; Laughlin and Bertozzi 2009; Tron et al. 2008; Svenson and Tomalia 2005; Li et al. 2007; Pieters et al. 2007; Agard and Bertozzi 2009; Rosen et al. 2009; Gao et al. 2005).

Click reactions are highly modular and stereospecific reactions with high yields due to high thermodynamic driving force (>20 kcal mol^{-1}) (Nandivada et al. 2007). The click chemistry does not represent a specific type of reaction, but rather defines a synthetic concept or framework that comprises a range of reactions, with different reaction mechanisms, but common reaction trajectories. The click chemistry can be divided into five main groups (Hein et al. 2008; Nandivada et al. 2007; Dondoni 2007; Roy and Touaibia 2007; Tron et al. 2008; Gauthier and Klok 2008): (1) cycloaddition of unsaturated species: 1,3-dipolar cycloaddition; (2) cycloaddition of unsaturated species: [4+2]-cycloaddition (Diels–Alder); (3) nucleophilic substitution/ring-opening reactions; (4) addition to carbon–carbon multiple bonds; and (5) carbonyl reactions of the non-aldol type.

Owing to limited space, we will restrict ourselves to the first type of reactions only, that is, the role of Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition. This approach presents a synthetic concept that lends itself perfectly to the controlled preparation of multifunctional materials with tailor-made properties, e.g. dendrimers. In general, all click reactions are easy to use, give high reaction yields, regiospecific, easy to purify, and do not require long reaction times. The Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes has emerged as the most popular click reaction by far (Fig. 5). Click chemistry found numerous applications across a wide variety of disciplines, including dendrimers, polymer chemistry,

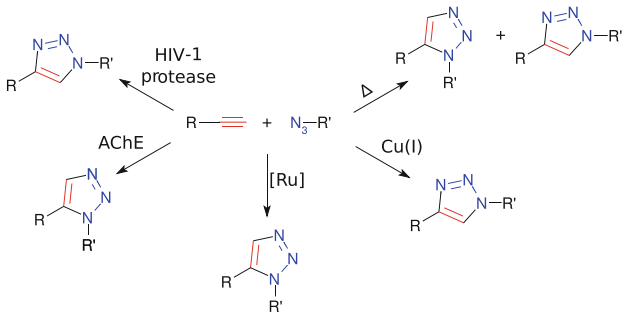

Fig. 5 Products of 1,3-dipolar cycloadditions between azides and alkynes (Tron et al. 2008)

material research, and the pharmaceutical sciences. In summary, the Huisgen-type azide–alkyne cycloaddition promoted by Cu(I) is a powerful tool in new strategies for the synthesis of various types of densely glycosylated molecular architectures, such as glycoclusters, glycodendrimers, glycopolymers, and complex glycoconjugates.

Unfortunately, there are a few important limitations. The most significant are biocompatibilities of copper, which serves as a catalyst of the reaction, and of 1,2,3-triazoles, which are in the products.

Since alkyne and azide substituents can be incorporated into a wide range of compounds, the potential of this reaction is very high (Tron et al. 2008; Pieters et al. 2007; Wang et al. 2007; Hong et al. 2009; Weiwer et al. 2009). Nearly a half of a century the reaction suffered from a lack of selectivity yielding a mixture of the 1,4- and the 1,5-regioisomers (Tron et al. 2008). Besides, this process requires heating and long reaction times to go to completion and the two regioisomers are at times laborious to separate using classical chromatographic procedures. Fortunately, two groups (Rostovtsev et al. 2002; Tornoe et al. 2002) independently found that this reaction can be accelerated up to 10 million times by the addition of Cu(I) salts. Moreover, the copper catalyst directs the formation to the 1,4-regioisomer only (Fig. 5), when the reaction is carried out at room temperature or with only moderate heating.

Quatrefoil-shaped star-cyclic polystyrene containing a polyhedral oligomeric silsesquioxane core was synthesized via the combination of atom transfer radical polymerization (ATRP) and click chemistry techniques (Ge et al. 2009). This model reaction confirmed that bimolecular click cyclization effectively occurred under highly dilute conditions.

The use of copper catalyst can be avoided when mono- or difluorinated cyclooctyne was used for click reaction (Johnson et al. 2008). The higher degree of fluorination led to faster reaction. This strain-promoted azide–alkyne cycloaddition proceeds very efficiently with high chemoselectivity even in in vivo applications, and therefore it is perfectly suitable for in situ crosslinking. Because no copper or ligand/base is required, there would be only two components of the reaction and, as a result, little extractable material. This work represents the first example of monitoring the kinetics of an in situ crosslinking process using the azide antisymmetric FTIR stretch.

Click chemistry was applied for synthesis of various nanoparticles with glycodendrimers attached on the surface (Martin et al. 2009; Wu et al. 2008; see "Dendrimers in nanoscience and nanotechnology"). This led to enhancement of biocompatibility. Kleinert et al. (2008) reported the synthesis of a broad variety of functionalized molecules for assembly on gold, allowing the formation of biologically relevant self-assembled monolayers (SAMs) by a modular approach: either utilizing 1,3-dipolar cycloaddition of alkynes and azides in solution or by "click on SAM".

Propargylated pentaerythritol phosphodiester oligomers (PePOs) were synthesized using a DNA synthesizer with a bis-propargylated pentaerythritol-based phosphoramidite. An azido L-fucose derivative was reacted under "click" chemistry conditions and microwave activation (Morvan et al. 2007). These compounds were active against *P. aeruginosa* (see also "Carbohydrate interactions of glycopeptide dendrimers"). Glyco oligonucleotide dendrimers, each with two mannose and two galactose residues, were effectively synthesized by two successive 1,3-dipolar cycloadditions (bi-click chemistry) (Pourceau et al. 2009). Because it is not possible for alkyne and azide functions to occur simultaneously on the same oligonucleotide since they would participate in an intramolecular reaction leading to cyclization, alkyne and azide functions borne by the same oligonucleotide must be introduced or generated successively in order to react specifically with azide and alkyne derivates by intermolecular cycloadditions. Thus, first an oligonucleotide was synthesized using an automated DNA synthesizer and alkyne or bromohexyl functions were introduced at its 3′- or 5′-end using the corresponding phosphoramidite derivatives. Second, a cycloaddition was done with an azidocarbohydrate derivative, introducing the first type of sugar on solid phase. Third, the bromohexyl groups were transformed to azidohexyl groups, which were finally conjugated with alkyne carbohydrate derivatives to introduce the second type of carbohydrate on solid support or in solution. This microwave-assisted bi-click chemistry (Pourceau et al. 2009) has proven high effectivity and can be applied for the synthesis of other heteroconjugates of oligonucleotides. For other applications of microwave-assisted synthesis of triazole-linked glycodendrimers by copper-catalyzed [3+2] cycloaddition see Joosten et al. (2005), Branderhorst et al. (2007, 2008), Pukin et al. (2007), and Pourceau et al. (2008).

The Cu(I)-catalyzed Huisgen cycloaddition of 2′-azidoethyl 2,3,4-tri-*O*-acetyl-*β*-xylopyranoside and alkynyl-terminated dendrimers provided a series of large

glycodendrimers containing 27, 81, and 243 terminal modified xylose branches (polyxylopyranoside dendrimers) (Camponovo et al. 2009). The molecular weight for G3-243-sugar dendrimer was > 130 kDa. This huge molecule was characterized by DOSY NMR, size exclusion chromatography (SEC), and dynamic light scattering. The chirality of sugar allows for the use of glycodendrimers in enantioselective catalysis.

Click chemistry was applied to the synthesis of mannose (α-Man)-containing glycodendrimers (the authors use the term neoglycoconjugates) with aliphatic-, aromatic-, and carbohydrate-centered architectures differing in structural characteristics, such as valency, topology, and nature of the linker (Perez-Balderas et al. 2009). The binding behavior (IC_{50} values) of these glycomimetics toward Concanavalin A (ConA) has been evaluated to determine the influence of the structural parameters. The same authors (Ortega-Munoz et al. 2009) used a series of different click-based strategies for efficient synthesis of multivalent, structurally diverse, heterogeneous neoglycoconjugates (glycodendrimers). This methodology is highly efficient and allows easy access to a series of mannose (α-Man)-containing glycodendrimers differing in their valency, nature of the constitutive sugars, nature of the scaffold, and length of the linker. Structure–activity relationships of binding affinities of these glycomimetics toward ConA were evaluated by ELLA and IC_{50} values were determined. Remarkably, the substitution pattern and the distance between the sugars are the more important parameters influencing the binding capabilities of these compounds.

Triazole glycocluster libraries (Dondoni and Marra 2006) were prepared via the Cu(I)-catalyzed 1,3-dipolar addition. Up to four 1,4-disubstituted 1,2,3-triazole rings with C-linked glycosyl fragments were constructed on various scaffolds via multiple cycloadditions of suitably polyfunctionalized calix[4]arene, adamantane, and benzene derivatives with ethynyl and azidomethyl *C*-glycosides. The cycloadditions occurred with high regioselectivity yielding exclusively the 1,4-disubstituted triazole ring in very high yield up to 98%. Wide scope of this approach together with high degree of efficiency constitutes a simple and practical means for the attachment of various sugar units to polyfunctionalized substrates.

Copper(I)-catalyzed azide–alkyne cycloaddition was applied for enhancement of structural diversity in phthalocyanine macrocycles (Chen et al. 2009a).

Roy's team synthesized a new family of glycodendrimer scaffolds containing 12 and 18 peripheral α-mannopyranosidic units by Cu(I)-catalyzed 1,3-dipolar cycloadditions using oligo-sulfurated dendritic scaffolds bearing alkyne functionalities and TRIS (tris(hydroxymethyl)aminomethane) derivatives (Chabre et al. 2008). It was shown that saturation of inhibitory potency was reached as a function of dendrimer scaffolding generation. Due to the limited surface of the denrimeric particle, individual sugars became inaccessible toward their protein receptors. Structures and valency were identified as critical factors in the optimization of individual binding interactions. In order to confirm those important observations authors are currently running biological studies to estimate their therapeutic potential, notably for the inhibition of adhesion of *E. coli* toward urothelial infections and other mannoside -binding proteins (Chabre et al. 2008).

The Cu(I) catalyzed azide–alkyne 1,3-dipolar cycloaddition was used (Ortega-Munoz et al. 2007) for efficient synthesis of fluorescent and non-fluorescent multivalent neoglycoconjugates (glycodendrimers). As a core, a well-defined glycopolymer, glycocyclodextrin, or glycocluster architecture displaying galactose or lactose epitopes has been chosen. Click multivalent neoglycoconjugates have the capability to act as synthetic activators mimicking the lipopolysaccharide in cell adhesion and stimulation of monocyte/macrophage cell lines. Gal and Lac-containing glycocyclodextrins were demonstrated to be the neoglycoconjugates with the highest adhesion and stimulation capabilities. These click-compounds are not limited to the activation of monocytes/macrophages, but also with potential for the development of therapeutics (Ortega-Munoz et al. 2007).

Other tetra- and hexavalent mannoside inhibitors of the pro-apoptotic, antiproliferative, and cell surface clustering effects of ConA have been synthesized by click chemistry using Cu(I)-catalyzed 1,3-dipolar cycloadditions with pentaerythritol scaffolds bearing either alkyne or azide functionalities and by Sonogashira coupling using pentaerythritol scaffolds bearing either alkyne or *p*-iodophenyl functionalities (Fortier et al. 2008). Their impact on membrane type 1-matrix metalloproteinase (MT1-MMP) functions in marrow-derived mesenchymal stromal cells (MSC) was studied. ConA-mediated changes in MSC morphology was reversed by the tetra- and hexavalent mannosides. The mannosides antagonized ConA-induced caspase-3 activity and proMMP-2 activation. They also reverse antiproliferative and pro-apoptotic impact of ConA on the MT1-MMP/glucose-6-phosphate transporter signaling axis. Since these glycoclusters specifically target MT1-MMP pleiotropic functions in cell survival, proliferation, and extracellular matrix degradation, they can be applied for anticancer therapy (Fortier et al. 2008).

Several oligomannoside dendrimers having a 100-fold increase in affinities toward *E. coli* were synthesized by Cu(I)-catalyzed 1,3-dipolar cycloadditions using pentaerythritol scaffolds bearing either alkyne or azide functionalities (Touaibia et al. 2007).

Non-hydrolyzable dendrimer containing 1,2,3-triazole-linked sialic acid derivatives was synthesized by click

chemistry (Weiwer et al. 2009) and studied as neuraminidase inhibitor. Micromolar IC_{50} value was observed, comparable to the known sialidase inhibitor *N*-acetyl-2,3-dehydro-2-deoxyneuraminic acid. In contrast to the natural *O*-glycosides, the non-natural *N*-glycosides of sialic acid are resistant to neuraminidase-catalyzed hydrolysis. A prevention of the release of new virions can be achieved by these neuraminidase inhibitors based on *N*-glycosides.

Very attractive approach for tuning of biological properties is called "in situ click chemistry" (Tron et al. 2008). Instead of synthesis of a collection of compounds and subsequent biological screening, it uses a mixture of reactants (dynamic library) and a tight reaction chamber—an enzyme (target), which selects appropriate enzyme inhibitor by allowing its formation in an enzyme active site. At room temperature, the enzyme brings alkyne close in proximity to an azide containing molecule; it decreases the high energetic barrier allowing the Huisgen reaction without copper catalysis to occur. By this way, reactants which fit correctly into the active site can form new potent ligands of the enzyme (Fig. 5) (Manetsch et al. 2004; Krasinski et al. 2005; Mocharla et al. 2004; Hu et al. 2008). Potent inhibitors of acetylcholinesterase (AChE) (Manetsch et al. 2004; Krasinski et al. 2005), HIV-1 protease (Whiting et al. 2006), matrix metalloproteinase (Hu et al. 2008), and carbonic anhydrase (Mocharla et al. 2004) have been discovered by this strategy. In the case of AChE (Manetsch et al. 2004; Krasinski et al. 2005), the enzyme facilitates the formation of 1,5-disubstituted triazoles, whereas HIV-1 protease has predilection for 1,4-disubstituted ones (Whiting et al. 2006). Thus, the enzyme can select one of two pathways of Cu-non-catalyzed reaction.

Finally, it is crucial to highlight the fact that click chemistry is a true interdisciplinary reaction which is able to help bridge the gap between chemistry and biology. The click chemistry links directly chemistry with biology (such as in the activity-based protein profiling assay) and can serve for tailored syntheses using biology (Tron et al. 2008; Pieters et al. 2007; Hein et al. 2008; Chabre and Roy 2008).

Purification and characterization of dendrimers

Since characterization of dendrimers by different types of methods, such as NMR, electron paramagnetic resonance (EPR), mass spectrometry, IR, Raman, UV–VIS spectrometry, optical rotation, circular dichroism (CD), synchrotron radiation-based circular dichroism (SRCD), fluorescence, X-ray diffraction, small-angle X-ray scattering (SAXS), small-angle neutron scattering, laser light scattering, atomic force microscopy (AFM), scanning tunneling microscopy (STM), optical tweezers, transmission electron microscopy (TEM), isothermal titration calorimetry (ITC), different chromatographic and electromigration methods (electrophoresis, capillary electrophoresis), time-resolved and non-linear optical spectroscopy, electron paramagnetic resonance, dielectric spectroscopy, differential scanning calorimetry, etc. have been deeply reviewed (Caminade et al. 2005; Shi et al. 2005b; Kehat et al. 2007; Niederhafner et al. 2008b; Martini and Ciani 2009; Biricova and Laznickova 2009; Shcharbin et al. 2009; Goodson 2005a, b; Shcharbin et al. 2007), only some of them will be mentioned.

Chromatographic methods

Use of chromatographic techniques for dendrimer separation and utilization of dendrimers as stationary phases for chiral resolution were reviewed (Kehat et al. 2007). The effect of multivalency on the performance of enantioselective separation media for chiral HPLC on polymer support with aliphatic dendrons was studied (Ling et al. 2002). The selectivity increases with the distance of the selector from the support core. The effective separation of enantiomers was achieved by these materials. The specific selectivity of the chiral stationary phase (CSP) was significantly higher for CSPs, built from perfect dendrons generated in solution, than for CSPs obtained by solid-phase divergent synthesis.

PAMAM-dendronized silica was used as a stationary phase for size-exclusion chromatography (Sakai et al. 2003).

Acosta et al. (2005) discovered two trends using dendronized stationary phases based on melamine. First, the higher generation of dendrons, the higher sequestering ability of the stationary phase was observed. Second, the divergently grown dendrons on silica possessed higher sequestering ability than that obtained by the focal-point immobilization of the dendrons pre-synthesized in solution.

Cason et al. (2008) described improved methodology for monitoring PAMAM dendrimers surface transformations and product quality by ultra performance liquid chromatography (UPLC). UPLC analysis was utilized for the first time as a methodology for monitoring PAMAM dendrimer surface transformations and product quality. The results in comparison with HPLC were found to provide a vastly improved analytical method for the characterization of dendrimer polydispersity and variance in a typical surface modification. The new UPLC procedures were used to monitor surface modification of G4 (PAMAM)-$(NH_2)_{64}$ dendrimer to produce biotinylated dendrimer conjugates. The use of UPLC increased the average number of theoretical plates by a factor of 7 and reduced retention times of analytes by 36%, while improving the resolution capability to discriminate surface variances in dendrimers. Besides, due to the reduced band spreading during the separation

process, the analytes are more concentrated at the point of detection. This enables lower injection volumes, improving the limit of detection by a factor of 100 (HPLC method for G4, PAMAMs, is 1.6×10^{-10} mol compared to 1.6×10^{-12} mol for UPLC).

This study indicates that UPLC, in comparison with HPLC, is an improved chromatographic method for the detection, purification, and separation of unmodified as well as surface-modified PAMAM dendrimers.

Quantification of peptide dendrimers was developed and validated using RP-HPLC method with UV detection. The assay was used for evaluation of skin permeation experiments (Mutalik et al. 2009).

For comparison of UPLC and HPLC (ruggedness and robustness) see Dejaegher and Heyden (2007), Toyo'oka (2008).

Electromigration methods

The purity assessment and purification of peptides, glycopeptides, and glycopeptide dendrimers have to be carried out using several methods based on different physical and physicochemical principles, such as HPLC, capillary electrophoresis, capillary electrochromatography, and other electromigration methods (Shcharbin et al. 2009; Biricova and Laznickova 2009; Caminade et al. 2005; Shi et al. 2005b; Kasicka 2003; Zamfir and Peter-Katalinis 2004; Peric and Kenndler 2003; Castagnola et al. 2002; Shi et al. 2005a, 2006c; Nilsson and Nilsson 2006). The use of methods that differ in the separation principle is necessary for checking of their preparative efficiency by other complementary analytical techniques, such as RP-HPLC and CE (Niederhafner et al. 2008b; Kasicka 2003; Zamfir and Peter-Katalinis 2004; Caminade et al. 2005; Shi et al. 2005b). Unfortunately, many authors confirm the final purity after separation by the same technique. It is necessary to use also a complementary method. Even combination of these techniques is not sometimes sufficient to assure removal of all by-products differing by only a single modification or deletion. Some of the above-mentioned methods (Nilsson and Nilsson 2006) use dendrimers as the pseudostationary phase (PSP) in capillary electrochromatography. Dendrimers can play a dual role. They can be both the subject and the object of separation. Dendrimers can be used as PSP for separation of other dendrimers. The use of dendrimers as PSPs for capillary electrokinetic chromatography has been reviewed (Peric and Kenndler 2003; Nilsson and Nilsson 2006). Addition of dendrimers to the background electrolyte leads to higher performance with respect to classical micellar electrokinetic chromatography separations. This has been attributed to a higher homogeneity of the dendrimer phase and a wider migration time window (Peric and Kenndler 2003). The separation is influenced by the size and charge of the dendrimers and by the composition of the electrolyte. The higher the concentration of dendrimer the better is the resolution achieved (Nilsson and Nilsson 2006).

Polyacrylamide gel- (PAGE) and capillary electrophoretic (CE) methods were used for analysis of PAMAM dendrimers and their derivatives (Brothers et al. 1998). In CE, generational separation was achieved up to the fifth generation of ammonia-core PAMAM dendrimers.

Negatively charged PAMAM succinamic acid dendrimers (PAMAM-SAH) (G1–G8) were analyzed by CE using a poly(vinyl alcohol)-coated capillary (Desai et al. 2008). The migration times were highly reproducible for all generations of dendrimers. A reverse trend in migration times for the PAMAM-SAH dendrimers (higher generations migrated faster than lower generation dendrimers) was seen in comparison to amine-terminated PAMAM dendrimers. A generational separation of lower generation (G1–G3) dendrimers was possible due to this reverse trend. Unfortunately, the same phenomenon prevented a sufficient separation of higher generations (G4–G5).

Affinity capillary electrophoresis (ACE) is useful for determination of binding constants of vasoactive intestinal peptide to PAMAM dendrimers (Dribek et al. 2007).

Dynamic coating CE was used for separation of compounds possessing amino groups (peptides, proteins, and polyamino compounds) (Sedlakova et al. 2006). This methodology removed common drawback of capillary zone electrophoresis (CZE), i.e. the sticking of these solutes with the capillary wall. Dynamic coating of the capillary allowed the separation of PAMAM dendrimers at pH 7.4, in contrast to CZE. This system allows the separation of 7 generations of PAMAM dendrimers (G0–G6). The dynamic coating agent (polyethyleneimine) also improves the separation at acidic pH.

G5 ethylenediamine-cored PAMAM dendrimers with different degrees of acetylation and carboxylation were synthesized (Shi et al. 2006a). They served as suitable models for studies of the effect of charge and the influence of dendrimer surface modifications on electrophoretic mobility (EM) and molecular distribution. Partially modified dendrimers displayed broader migration peaks than those of fully surface functionalized or totally non-functionalized ones. The higher the surface acylation of both PAMAM acetamides and PAMAM succinamic acids the less is the EM observed. Since this phenomenon has shown non-linear behavior, a complex migration activity in CE separations cannot be solely explained by charge/mass ratio changes.

Ethylenediamine-core PAMAM succinamic acid dendrimers were investigated (Shi et al. 2005c). PAGE revealed that the higher generation of dendrimer the less relative mobilities were observed.

PAMAM dendrimers with acetamide, hydroxyl, and carboxyl surface group were synthesized from ethylenediamine core G4 and G5 with primary amine groups on the surface (Shi et al. 2006b). The products were purified with dialysis. PAGE and CE electropherograms have shown the purity, charge distribution, and electrophoretic mobility of the compounds, whereas SEC and MALDI-TOF–MS provided the average molar mass and the individual mass fractions, respectively. SEC in combination with potentiometric titration afforded quantitative evidence of the degree of the functional group substitution. NMR techniques confirmed the changes in dendrimer surface functionalization. This work is an example for the comprehensive characterization of PAMAM dendrimer and their surface functionalization.

A carbosilane dendrimer was used as the coating for the capillary column (Shou et al. 2008b). Since the coated column suppressed the absorption of Si–O to basic substances, adenine, adenosine and 6-furfurylaminopurine were totally separated in 20 min. The best separation effect was observed for twice coated capillary.

The same authors (Shou et al. 2008a) prepared a new chiral capillary electrophoresis column coated with carbosilane dendrimers with β-cyclodextrin. Chlortrimeton, promethazine, and benzedrine were used as separation model targets. The modified column was especially effective in separating chlortrimeton enantiomers.

Polycationic amphiphilic cyclodextrins self-assemble in the presence of plasmid DNA (pDNA) to provide homogeneous, stable nanoparticles (CDplexes) with the size 70–150 nm that fully protect pDNA from the environment (Diaz-Moscoso et al. 2009). They were applied as a tool for gel electrophoresis.

Mass spectrometry

Mass spectrometry (FAB-MS, MALDI-TOF MS, ESI–MS, MS/MS tandem MS, QqTOF quadrupole–quadrupole time-of-flight, etc.) is the most important methodology for structure elucidation and determination of glycopeptides and glycopeptide dendrimers (Caminade et al. 2005; Dell et al. 2000, 2008; Lehmann et al. 2000; Haslam et al. 2003; Krokhin et al. 2004; Schalley et al. 2006; Morelle and Michalski 2005). This methodology provides the molecular weight of the product and even it can help in identification and quantification of certain impurities. Moreover, mass spectrometry can be used for studies of conformation and dynamics of biomolecules including glycopeptides (Kaltashov and Eyles 2005).

ESI–MS-based methods can be useful for the determination of K_a values of anion complexation by glycocluster thioureamethyl calix[4]resorcarenes (Oshovsky et al. 2004). It is an excellent method for rapid and quantitative determination of the complex behavior of a host toward a variety of guests.

In some cases, one cannot obtain the correct MALDI-TOF MS spectra (Niederhafner et al. 2008a; Baigude et al. 2003, 2004). The results of the MALDI-TOF–MS measurements were dependent on the molecular mass. As the mass increased (10.8–13.3 kDa) a considerable difference was seen between the found (13357.37 Da) and calculated (13264.95 Da) masses (Baigude et al. 2003, 2004).

"Fake defects" were observed in ESI and MALDI mass spectra (Schalley et al. 2006; Baytekin et al. 2006; Felder et al. 2005). In the first case, ESI–MS of PPI dendrimers showed a high abundance of new type of defects, which could not be confirmed by ^{1}H, ^{13}C NMR spectra and MALDI MS. In the second example, ESI–MS of dendrimers with sulfonamide groups in their periphery have shown high sample purity; however, MALDI MS produces signals for defects that seem to be generated during synthesis. This was explained by thermal reactions during ionization within the matrix and not synthetic problems. Mass spectral data of dendrimers must be interpreted and evaluated with care, keeping in mind that sometimes false negative data can be obtained (Niederhafner et al. 2008b).

Standard MALDI-TOF–MS is useful for molecular weight determination of ultra-high mass compounds (Muller and Allmaier 2006), such as immunoglobulin M and G10 PAMAM dendrimer.

Glycoscreening in biomedical research by capillary electrophoresis-mass spectrometry has been reviewed (Zamfir and Peter-Katalinis 2004). Mass spectrometry was used for screening of molecular recognition and self-assembly in supramolecular chemistry of dendrimers (Schalley 2001). This review provides many examples of the use of mass spectrometry as an analytical tool for the determination of molecular weight of non-covalently bound supramolecular aggregates.

Amphiphilic dendrimers are capable to form environment mimicing normal and reverse micelles depending on the solvent properties (Azagarsamy et al. 2009a). Their ability to sequestrate a guest molecule was investigated for extraction of compounds of interest. The reverse micelle-like assemblies formed by these materials with buried carboxylate groups possessed a hydrophilic environment for positively charged molecules in their interior. This is a suitable tool for extraction and preconcentration of positively charged peptides from aqueous solutions. The extracted peptides can be directly analyzed by MALDI-MS with increased detection limits down to 500 pM from volumes as small as 250 μL.

A complex dendrimeric contrast agent Gadomer has been developed and validated by a CE and MALDI-TOF–MS methods (Vetterlein et al. 2006). CE was capable to separate Gadomer 24 from related dendrimers and from

impurities of lower molecular weight. This can serve as a strategy for the quality assurance and quality control of the complex dendrimeric drug candidate Gadomer. MS data obtained by MALDI-TOF–MS measurements were confirmed by CE-ESI-TOF–MS measurements (Vetterlein et al. 2007). Moreover, high-resolution Fourier transform ion cyclotron resonance-MS (FTICR-MS) with/without CE provides mass spectra of high mass accuracy and resolution of various impurities and related dendrimers present in low concentrations in several Gadomer batches.

Dendrimers with cyclic core

RAFTs, TASPs, and analogous cyclic structures

The template-assembled synthetic protein (TASP) was introduced by Mutter as a simple system for modeling and exploring the underlying principles that govern a protein's folded structure (Mutter et al. 1988; Mutter and Vuilleumier 1989). This methodology was a milestone of de novo protein science. The first TASP obtained by the covalent ligation of de novo peptides to a peptidic template with formation of a four-helix bundle mimetic was described a year later (Mutter and Vuilleumier 1989). A key feature of this template (Renaudet 2008; Grigalevicius et al. 2005) (Fig. 6) is the formation of two faces with regioselectively functionalizable amino acid side chains. Due to the presence of selectively addressable side chains, these templates are also called regioselectively addressable functionalized templates (RAFTs) (Boturyn et al. 2008). A structural prototype of RAFTs has been synthesized and crystallized (Peluso et al. 2001). The three-dimensional structure of aromatically substituted RAFTs was determined by X-ray diffraction and NMR spectroscopy. Tetravalent template-assembled glycopeptides, namely regioselectively addressable functionalized templates (RAFTs), were prepared by the oxime ligation (Renaudet and Dumy 2003; Grigalevicius et al. 2005). Derivatives with four β-Glc, β-Gal, β-GalNAc, β-Lac, α-Glc, α-Gal, α-GalNAc, and α-Man, respectively, were isolated by semipreparative HPLC in almost 80% yield. A tetramannosyl-RAFT with ConA was investigated by fluorescence anisotropy-based recognition assays. Nearly 20-times improvement of IC_{50} was achieved in comparison with methyl α-mannopyranoside.

Synthesis and biological evaluation of clicked curcumin and clicked KLVFFA conjugates as inhibitors of β-amyloid

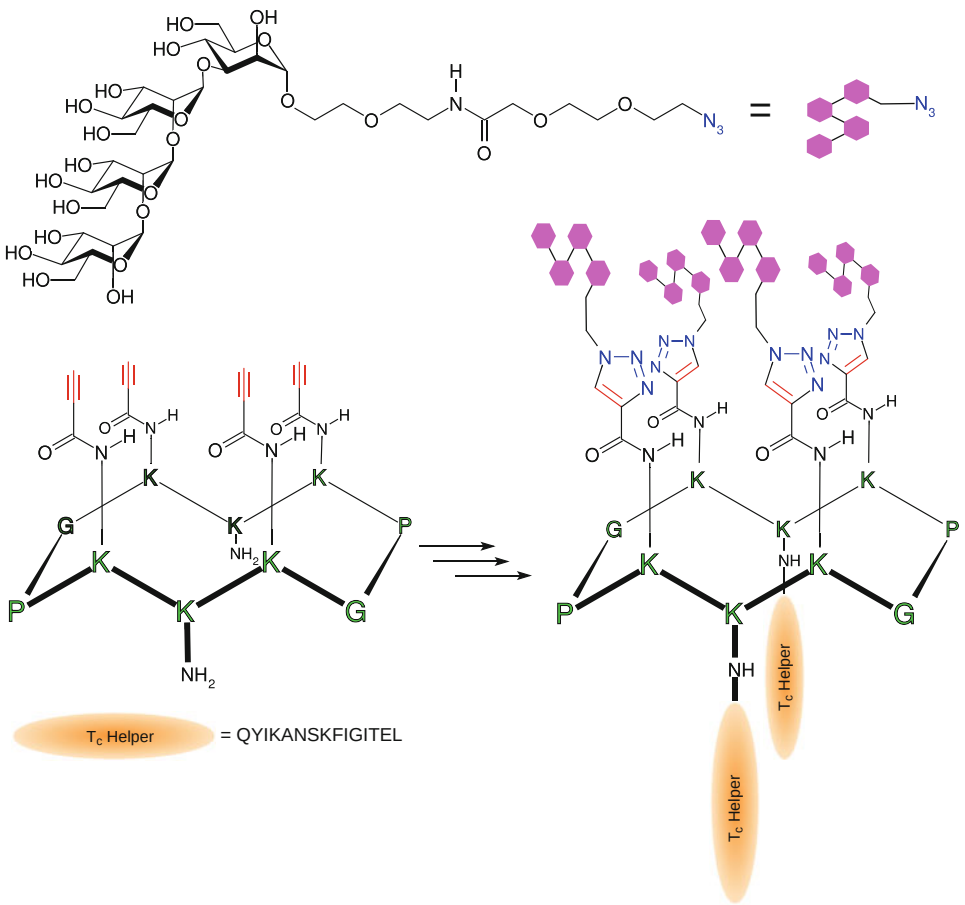

Fig. 6 Synthesis of RAFT-based anti-HIV vaccine by click chemistry (Wang et al. 2007)

fibril formation were described (Ouberai et al. 2009). Decapeptide TASP was used as cyclic template. On the upper face of the cyclopeptide scaffold two copies of the KLVFFA peptide or two copies of the curcumin molecule were located. Four Arg residues were on the lower face.

On-bead synthesis of chemoselectively template-assembled multivalent neoglycopeptides was described (Renaudet and Dumy 2006). Aminooxylated carbohydrates (β-Lac-ONH_2, α-GalNAc-ONH_2, and α-Man-ONH_2) have been prepared as carbohydrate-based recognition elements and conjugated with RAFTs using oxime ligation. These RAFT-containing beads were used for recognition and detection of lectins. Signal amplification was achieved using labeling of lectins with horseradish peroxidase.

A new strategy for the synthesis of glycopeptide–oligonucleotide conjugates was described. It utilizes a cyclodecapeptide scaffold (RAFT) containing 5 Lys residues for anchoring the lactose cluster and the oligonucleotide through sequential oxime ligation (Singh et al. 2005). The duplex formed by the oligonucleotide glycocluster with the complementary sequence has shown its stability during thermal denaturation and CD experiments. The binding interactions of the oligonucleotide glycocluster with specific lectins from *Arachis hypogaea* (peanut) were significant. Non-specific binding with ConA was not observed.

RAFTs conjugated with ferrocene units served as redox probe, which could be attached on golden surface of electrodes (Devillers et al. 2006).

TASP was used also for synthesis of combinatorial libraries (Dulery et al. 2008) (see "Dendrimeric libraries").

Sherman's group uses the TASP concept more freely (Huttunen-Hennelly and Sherman 2008; Freeman et al. 2009). They synthesized so-called caviteins, where macrocyclic peptide template is substituted with a cavitand—macrocyclic mostly aromatic system. Cavitands can be readily linked to peptides and form caviteins (the combination of **cavit**and + prot**ein**). According to X-ray studies, one can predict that caviteins will have similar advantages as corresponding peptide-based TASPs.

The same group reported preparation and solution study (Nikan and Sherman 2008, 2009) of template-assembled synthetic G-quartets (TASQs). These G-quartet baskets extract cations of different sizes and valencies. Isolated G-quartets are formed with small cations, such as Na^+ and Sr^{2+}, and dimeric structures with larger cations, such as Cs^+.

Some authors use the term cyclic peptide scaffolds instead of TASPs or RAFTs (Krauss et al. 2007; Chabre and Roy 2008). Di- and trivalent glycopeptide mimics of the HIV 2G12 epitope bound to cyclic peptide scaffold (TASP) have been prepared (Krauss et al. 2007) and their binding characteristics were tested. The TASP, unrelated to gp120 peptide sequences, was attached with aspartate linkages to two or three copies of the high-mannose glycan, $Man_9GlcNAc_2$. Biacore assay has shown that the higher the valency the higher is the binding affinity for 2G12.

A new class of template-assembled oligomannose clusters mimicking the epitope of the HIV-neutralizing antibody 2G12 was synthesized (Fig. 6) (Wang et al. 2007). The branched oligomannose arms were successfully assembled on decapeptide RAFT by means of the Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides to alkynes. On one face four $Man_9GlcNAc_2$ units were bound. Two T-helper epitope peptides were introduced on the other face of the template. Their interactions with human antibody 2G12 were studied using surface plasmon resonance (SPR) technology. The novel synthetic RAFT-based glycoconjugates stand for a new type of immunogen that can raise carbohydrate-specific neutralizing antibodies against HIV-1.

Additional route to oxime and sulfur ligations for synthesis of TASPs and RAFTs was explored by Diederichsen group (Avrutina et al. 2009). It is based on a Cu(I) catalyzed azide–alkyne cycloaddition using a cyclic peptide template as a versatile conjugation scaffold. This method provides effective coupling of unprotected peptide monomers in water at room temperature within short reaction times. Since the ligands are displayed in an oriented manner, multivalent interactions with given target molecules can take place. This method allows also an orthogonal coupling for the incorporation of fluorescent labels or radioligands.

TASP and RAFT molecular engineering approaches allow for a rational design of complex systems with tailor-made properties and pave the way to more sophisticated applications using bottom-up design of dendrimers and nanostructures (Boturyn et al. 2008).

The synthesis and biological applications of RAFTs and TASPs were deeply reviewed (Tuchscherer and Mutter 2004, 2005; Boturyn et al. 2008).

Carbopeptides, carboproteins, and octopus glycosides

Carbopeptides represent the use of carbohydrates as potential templates (cores) for de novo design of peptide and protein models (Jensen and Brask 2005). We can say that carbopeptides are TASPs on carbohydrate templates. The term carboproteins is used for protein models on carbohydrate templates. The use of sugar amino acids (sometimes the term carbohydrate amino acids is also used) multiplies the variability of the template approach. For reviews on carbopeptides see Jensen and Brask (2002, 2005), Niederhafner et al. (2008a), Tofteng et al. (2007), Brask et al. (2003b), Jensen and Barany (2000), Brask and Jensen (2000).

A carboprotein with 4-α-helix bundle structure, as well as a carbopeptide with a truncated peptide sequence

(Thulstrup et al. 2005) were studied by SRCD. The use of synchroton radiation (SR) enabled CD measurements in the vacuum ultraviolet region down to 168 nm in D_2O and 160 nm in 2,2,2-trifluoroethanol (TFE).

The same authors (Hoiberg-Nielsen et al. 2008) described the synthesis and structural studies of α-helical bundle carboproteins derived from methyl α-galactopyranoside (Gal*p*) and methyl α-altropyranoside (Alt*p*), which were assembled from the corresponding carbohydrate templates and one amphiphilic hexadecapeptide sequence. The solution structures of the carboproteins were analyzed by means of SAXS and SRCD. Chosen templates induced formation of 3+1 helix bundle instead of expected 4. The template significantly influenced structure of the compound in solution. In solution, folding of carboproteins can be controlled by subtle variations of template distance-geometry. Significant increase of thermostability was achieved by template assembly.

Functionalized de novo designed 8–16 kDa model carboproteins were synthesized and their metal ion-binding and esterase activity were studied (Tofteng et al. 2007).

Glucuronic acid derivatives were used as branching units for the synthesis of glycopeptide mimetics (Rockendorf and Lindhorst 2004).

Another sort of carbohydrate-centered glycodendrimers with carbohydrate cyclic core is called "octopus glycosides" (Fig. 7) (Dubber and Lindhorst 1998, 2001; Rockendorf and Lindhorst 2001; Lindhorst 2002; Dubber et al. 2006b; Kohn et al. 2004). Other authors called octopus glycosides by various names, such as multivalent neoglycoconjugates (Ortega-Munoz et al. 2007, 2009), glycoclusters (Ortega-Munoz et al. 2007, 2009), carbohydrate-centered architectures (Perez-Balderas et al. 2009), and functionalized carbohydrate-centered glycoclusters (Sperling et al. 2007).

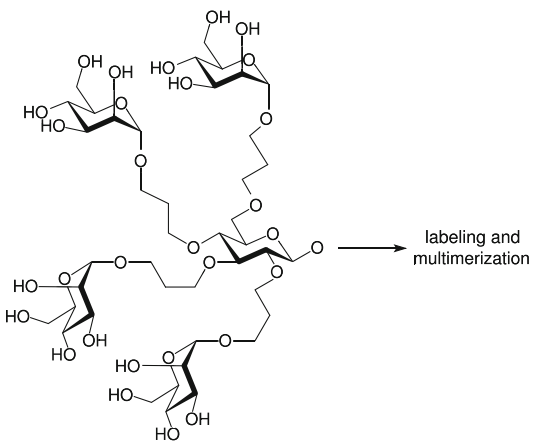

Fig. 7 Typical example of octopus glycoside with glucose core (Sperling et al. 2007)

Basic principles for synthesis of octopus glycosides were described (Dubber and Lindhorst 1998). They serve as core molecules for the construction of glycoclusters and carbohydrate-centered dendrimers.

Octopus glycosides based on octaamino-functionalized trehalose core were used for the synthesis of carbohydrate-centered PAMAM dendrimers and thiourea-bridged glycoclusters (Dubber and Lindhorst 2001). The glycoclusters contained α-mannose.

Tethered cluster mannosides with glucose as a core and 6-aminohexane aglycone as an anchor were prepared (Dubber and Lindhorst 2000; Dubber et al. 2006a). These compounds serve for studies of carbohydrate–protein interactions and for affinity chromatography.

Activity of carbohydrate-centered glycoclusters as ligands for ConA (a mannose specific lectin) was studied by a competitive ELLA. Immobilized yeast mannan served as the reference ligand. Both α- and β-glucopyranose was used as a core (Kohn et al. 2004). The activity depended on the spacer between the mannose units and the core. Dendrimers with 1-thiomannose were active. Replacement of 1-thiomannose with α-mannopyranosylthioureido units virtually abolished any activity, with a dramatic decrease of binding affinity. The diastereomeric 1-thiomannose-coated α- and β-glucopyranose were potent ligands for ConA. The influence of glycocluster valency to the biological activity was also studied.

The same group (Sperling et al. 2007) described a synthesis of dodecavalent octopus neoglycoconjugate using squaric acid diester-mediated coupling and tris(2-aminoethyl)amine as a core. The dendrimer with 12 α-mannose units was tested for anti-adhesive properties which allow detection of the adhesion of type 1 fimbriated *E. coli* to a mannan-coated polystyrene surface. The relative inhibitory potency (RIP) of this dendrimer was 190 in comparison with methyl α-mannoside (RIP = 1). See also Dubber et al. (2006b).

To simplify synthesis, peptide chemistry was used for the linking step instead of glycosylation techniques. Stability against glycosidases was investigated by HPLC. No degradation with β-glucosidase from almonds was observed over several hours (Dubber et al. 2006a).

Octopus glycosides prepared by native chemical ligation were tested as synthetic vaccine against group A streptococcal infection (Zhong et al. 2009). The use of suitable template which directs the attached peptides to form a well-defined tertiary structure increased their antigenicity. The conjugation of immunostimulatory lipids has been demonstrated as a potentially safe method for self-adjuvanting human vaccines.

Click reaction of methyl-2,3,4,6-tetra-*O*-propargyl-β-galactopyranoside with 2-azidoethyl glycosides of lactose and *N*-acetyllactosamine was used for synthesis of water

soluble and lectin-recognizable carbohydrate-centered glycoclusters (octopus glycosides) (Gao et al. 2005). The binding of a plant lectin RCA_{120} was inhibited by the glycoclusters 400-fold more strongly than with free lactose.

The topic of octopus dendrimers has been reviewed (Roy and Touaibia 2007), however, the terms carbohydrate centered glycocluster, and sugar scaffolds were used instead (Fig. 8).

Inositol-based dendrimers

A synthesis of functionalized glycopeptide dendrimers based on the *scyllo*-inositol scaffold, where the directionality the number, and density of the terminal α-Man can be controlled, was described (Lee et al. 2005b). The amino groups of the glycopeptide dendrimer reacted with 2,3,4,6-tetra-*O*-acetyl-α-mannosyl isothiocyanate. In this way, depending on the generation, α-Man was bound to *scyllo*-inositol scaffold with valencies 6 and 12.

The carriers of doxorubicin, an anticancer antibiotic, were designed and synthesized using dimeric inositol scaffolds (Maiti et al. 2007). They were prepared by linking two units of *myo*- or *scyllo*-inositol with a carbonate or amide bond. The scaffold was then functionalized by means of peracylation with *ω*-aminocarboxylate derivatives of varying length and the multiple units of the guanidine were introduced. These carriers have interesting cellular uptake characteristics and unique in vivo distribution. In terms of the uptake efficiency and amount of transporter, the amide-linked dimeric inositol carriers were superior to Arg_8 and Arg_9. Doxorubicin was efficiently delivered into mouse brain by these carriers.

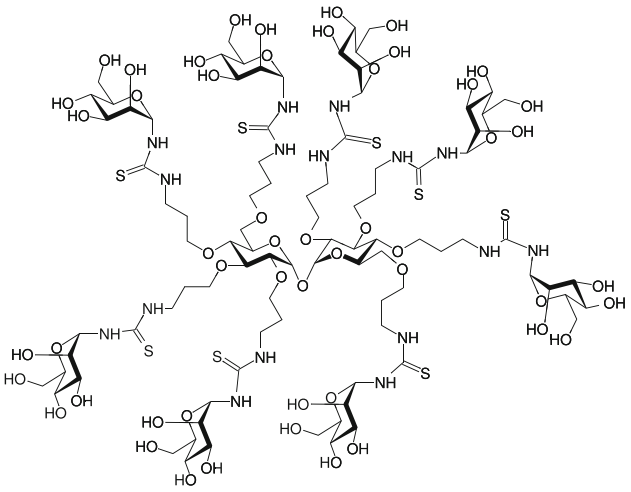

Fig. 8 Example of 8 α-Man attached to α,α-trehalose-based octopus glycoside with direct thiourea linkages (Roy and Touaibia 2007)

Cyclodextrins

Synthesis, physicochemical properties, and biological activities of different sorts of peptide and glycopeptide dendrimers based on different sorts of cyclodextrins (α, *β*, and *γ*) have been reviewed (Wang and Kaifer 2009; Niederhafner et al. 2008a; Roy and Touaibia 2007; Fernandez et al. 2006; Chabre and Roy 2008; Jensen and Brask 2005). CDs are cyclic oligosaccharides typically containing six, seven, or eight (α-, *β*-, or *γ*-CD, respectively) α-1,4-linked glucopyranosyl residues. Their ability to form inclusion complexes with hydrophobic guests within their largely hydrophobic cavities has a long recorded history (Frampton and Anderson 2007). The binding interactions between dendrimers and two types of molecular hosts, i.e. cyclodextrins and cucurbit[*n*]urils have been reviewed (Wang and Kaifer 2009). These interactions allow formation of supramolecular dendrimers from monomolecular dendron species. In this case, cyclic core is attached to the dendrimer by non-covalent interactions. The thermodynamics of these host–guest reactions varies depending on dendrimer size. Synthesis of double cavity cucurbituril hosts, which are suitable as dendrimeric core, was described (Nally and Isaacs 2009).

Many CD "pearls" thread spontaneously onto polymer "strings", such as poly(ethylene)glycol (PEG), poly(propylene)glycol, and poly(tetrahydropyran). The formed supramolecules are called pseudopolyrotaxanes (Nelson and Stoddart 2003). Derivatization of the termini led to rotaxanes, which can serve for enhanced drug and gene delivery (Li and Loh 2008; Yang et al. 2009a).

CD-based dendrimers represent another possibility of defined stereoselective structure endowed with cluster (multiplication) effect. Many examples of mono- and multivalent *β*-CD neoglycoconjugates of *β*-Glc, *β*-Gal, α-Man, *β*-Fuc, *β*-L-Fuc, cellobiose, lactose, GM3 trisaccharide, etc. were prepared by chemical or chemoenzymatic synthesis (Fernandez et al. 2006; Andre et al. 2004b; Benito et al. 2004).

Polycationic amphiphilic cyclodextrins (paCDs) were used as gene delivery systems (Diaz-Moscoso et al. 2009). The synthesis used a chemoselectivity of primary versus secondary hydroxyl groups on the CD torus to regioselectively decorate each rim with cationic elements and lipophilic tails, respectively. Their highly symmetrical architecture is reminiscent of both cationic lipids and cationic polymers, the two most prominent types of non-viral gene vectors. The modular synthesis of monodisperse paCDs is suitable for structure–activity relationship studies.

Ligands for ConA based on *β*-cyclodextrin were studied as a supramolecular control of oligosaccharide–protein interactions (Fig. 9) (Smiljanic et al. 2006).

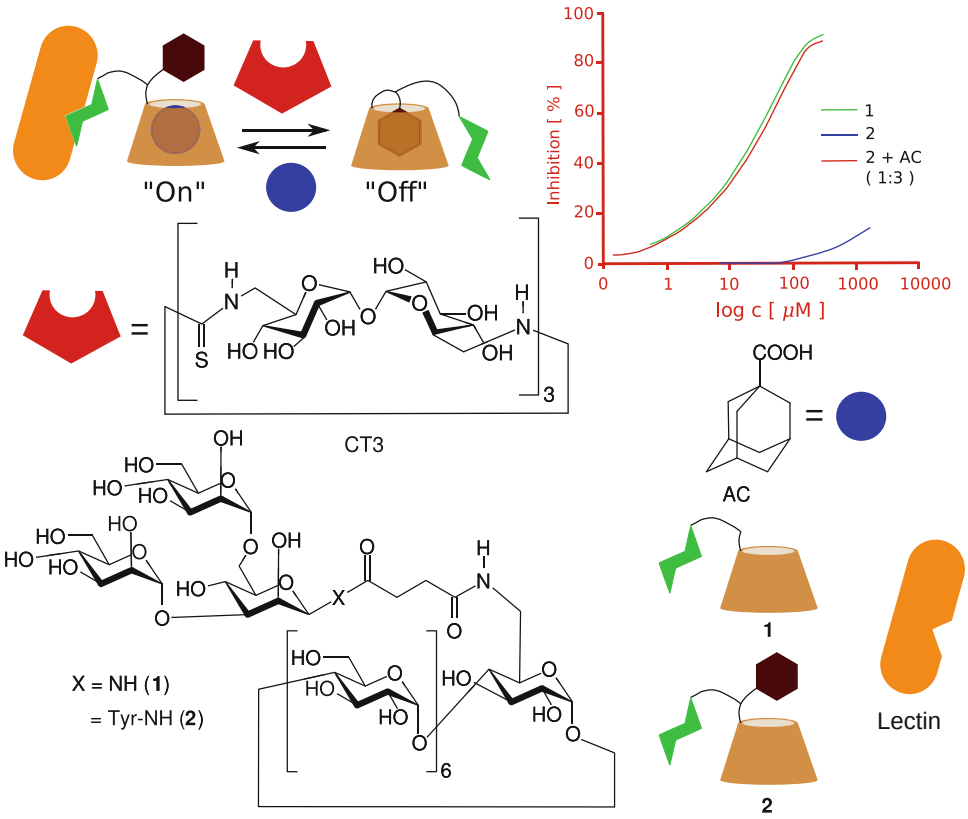

Fig. 9 Schematic representation of "on/off" switching of carbohydrate–lectin interactions via allosteric binding (Smiljanic et al. 2006)

Well-defined dendrimers with β-CD cores and poly(2-(dimethylamino)ethyl methacrylate) arms (P(DMAEMA)), and P(DMAEMA)-block-poly(poly(ethyleneglycol)ethyl ether methacrylate) were prepared via ATRP from the bromoisobutyryl-terminated β-CD core (Xu et al. 2009). These dendrimers condensed plasmid DNA (pDNA) into 100−200 nm size nanoparticles. They have much lower cytotoxicity and higher gene transfection efficiency in comparison with high molecular weight P(DMAEMA) homopolymers. The unique star-shaped architecture involving the CD core led to enhancement of the gene transfection efficiency.

Click non-fluorescent and fluorescent multivalent glycocyclodextrins were studied as synthetic activators in cell adhesion and stimulation of monocyte/macrophage cell lines (Ortega-Munoz et al. 2007).

Supramolecular chemistry of carbohydrate clusters with CD core has been reviewed (Vargas-Berenguel et al. 2007; Frampton and Anderson 2007), including site-specific drug delivery systems.

De novo synthesis of a new family of glyconanocavities based on α,α-trehalose building blocks, namely cyclotrehalans (Fernandez et al. 2006), and their complexing properties have been described.

Inclusion complexation takes part between β-CD-ended linear poly(*N*-isopropylacrylamide) (β-CD-PNIPAM) and Frechet-type benzyl ether dendron with an azobenzene group (Zou et al. 2009). The formed non-covalently connected amphiphiles, self-assemble into vesicles in water. The alternation of visible and UV irradiation led to optical switching of the assembly and disassembly. This is caused by the isomerization of the azo groups and their ability to complex β-CD. These photoresponsive supramolecules were sensitive to heat stimuli resulting in reversible aggregation and disaggregation.

A novel series of multivalent polycationic β-CD "click clusters" with discrete molecular weight have been synthesized and tested as therapeutic pDNA carriers (Srinivasachari et al. 2008). The synthesis was carried out by linking a per-azido-β-CD core (**3**) to oligoethyleneamine alkyne (**4**) using click coupling chemistry. According to a variety of characterization techniques, the final CD-dendrimers (Fig. 10) were completely substituted and deprotected. These macromolecules were highly water soluble, and most of them were found to condense and protect pDNA like the viral delivery vehicles. β-CD with the longest dendron arms (**6d**) and (**6e**) were the most effective delivery vehicles. The significance of these compounds for clinically viable drug delivery vehicles is discussed.

Calix[4]arenes, resorcarenes, and cavitands

The syntheses of hyperbranched and dendrimeric calix[4]arenes and thiacalix[4]arenes were reviewed (Niederhafner

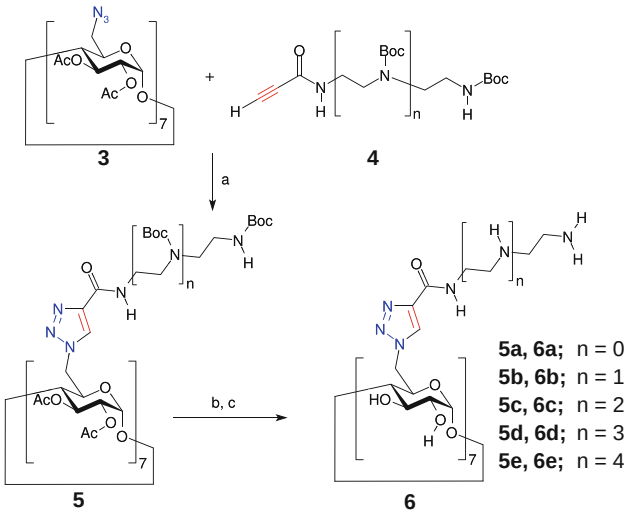

Fig. 10 Synthesis of *β*-CD based dendrimers prepared by click chemistry. **a** $CuSO_4 \cdot 5H_2O$, sodium ascorbate, tBuOH: H_2O (1:1), 70°C. **b** NaOMe/MeOH, pH = 9, RT. **c** 4 M HCl/dioxane

et al. 2008a; Baklouti et al. 2006a, 2006b; Roy and Touaibia 2007; Roy 2003; Martos et al. 2008; Kim et al. 2009; Rudzevich et al. 2009a, b).

C-glycoside clusters on calix[4]arene, adamantane, and benzene scaffolds were described (Dondoni and Marra 2006) (for more details see also "Click chemistry").

Syntheses and applications of hyperbranched calixarene-based dendrimers as fluorescent probes have been reviewed (Kim et al. 2009).

Chemical, physical, and physicochemical properties (NMR spectroscopy, mass spectrometry) of dimerization and self-sorting of tetraurea calix[4]arenes including examples of their dendritic assemblies have been described (Rudzevich et al. 2009a, b; Braekers et al. 2008).

Starting from amino acid-calixarene building blocks, calix[4 or 8]arene-based glycoconjugates exposing terminal *N*-acetyl-glucosamine clusters were synthesized (Consoli et al. 2004). These glycosamino acid-calixarenes possessed lectin-binding ability and amplified inhibitory effects on erythrocyte agglutination induced by wheat germ (*Triticum vulgaris*) agglutinin (WGA). Activity of these inhibitors can be modulated by the nature of the spacer and by the shape and rigidity of the calixarene skeleton.

Self-assembly of programmed building blocks into structurally uniform dendrimers was studied (Rudzevich et al. 2005). Selective and independent dimerization of tri- and tetraurea derivatives leads to dendritic assemblies uniform in size and structure (Fig. 11). Dendritic structures with molecular masses of about 25 kDa were obtained. The uniform assemblies were proved by dynamic light scattering and ^{1}H and ^{1}H DOSY NMR experiments.

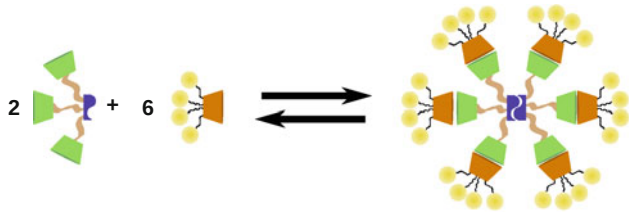

Fig. 11 Supramolecular dendrimer formed by self-assembly of calixarene building blocks (Rudzevich et al. 2005)

Analogous structures, such as resorcarenes and cavitands were used as cores of various dendrimers (Mendrek and Trzebicka 2009; Freeman et al. 2009; Huttunen-Hennelly and Sherman 2008; Nikan and Sherman 2008, 2009).

Calix[4]arenes can be located also as terminal groups on the dendrimer. Self-assembled supramolecular nanocarrier was prepared from hyperbranched polyethylenimine (HPEI) and an amphiphilic calix[4]arene (AC4) (Lou et al. 2009). The four AC4 phenol groups form supramolecular entity by non-covalent interaction with HPEI. The cationic water-soluble dye methyl blue (MB) and the anionic water-soluble dye methyl orange (MO) were sequestrated by supramolecular complex of HPEI-AC4. HPEI-AC4 buried the anionic water-soluble MO guests into the HPEI core. Simultaneously, the cationic MB molecules were captured by the outer AC4 shell. The amount of MO and MB captured by HPEI-AC4 can be governed by varying the ratio of amino groups of HPEI to hydroxyl groups of AC4.

A new class of a carbosilane dendrimer architecture was described, to which polycalix[4]arene hosts have been attached (Lambert et al. 2009). Each dendrimeric branch terminates with a calix[4]arene entity. The synthesis, characterization, and metal capture were studied for the G0 and G1 dendrimers contained on the periphery 4 and 12 calix[4]arenes, respectively.

Porphyrins

Other examples of cyclic cores are porphyrins. This class of compounds possesses various interesting properties, such as complexation of metals, light harvesting, interaction with nucleic acids, etc. There are many ways how porphyrin-containing dendrimers can be built up (Li and Aida 2009; Maes and Dehaen 2009) (Fig. 12).

Dendritic porphyrin-based nanoprobes were used for precise detection of pH inside large unilamellar vesicles (Leiding et al. 2009).

Cytochrome *c*-binding proteo-dendrimers with porphyrin core were capable to prevent mitochondrial apoptosis in HeLa cells (Azuma et al. 2009). It was attributed to their electrostatic interactions with cytochrome *c* in aqueous solutions. They can also serve as promising candidates for certain apoptosis-mediated diseases, such as hepatitis.

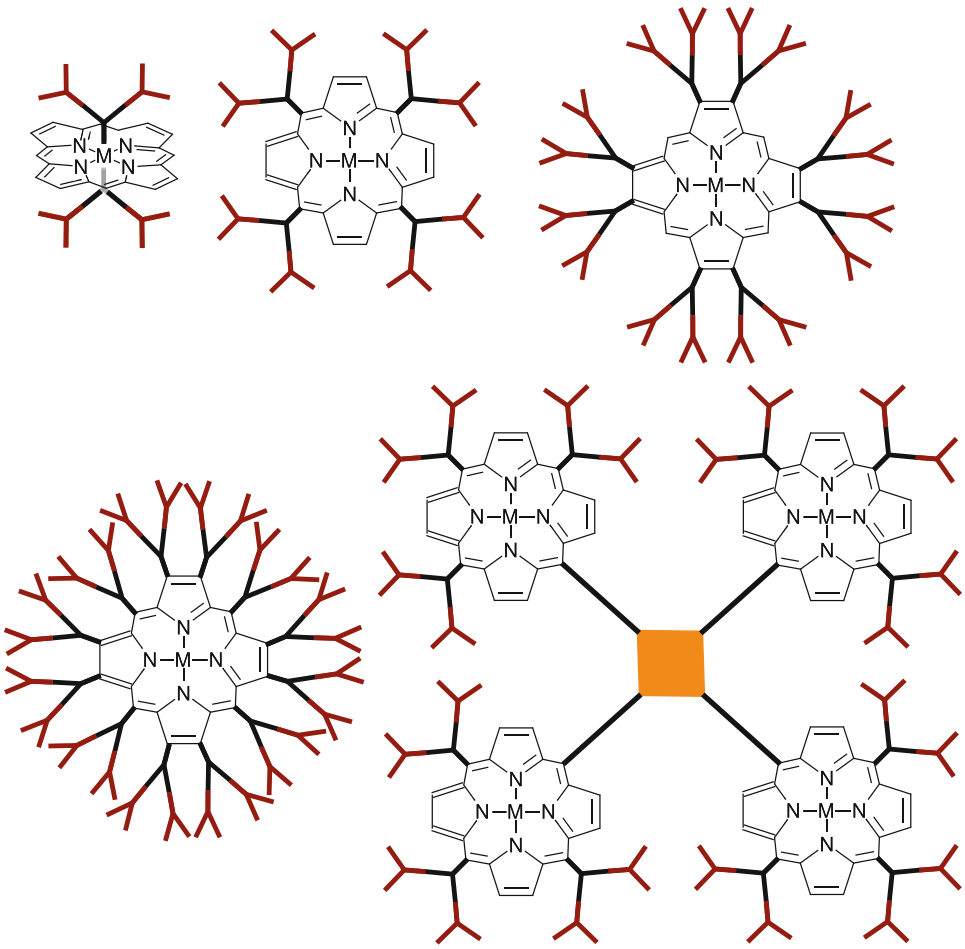

Fig. 12 Different sorts of porphyrin dendrimers (Li and Aida 2009; Maes and Dehaen 2009)

Dendritic single donor–acceptor conjugate with fullerene C_{60} based on porphyrin core was investigated as artificial mimicry of the primary events in photosynthesis, such as light harvesting, unidirectional energy transfer, charge transfer, and charge-shift reactions (Schlundt et al. 2009). Due to the flexibility of the linkers that connect the fullerene C_{60} with the metal-free porphyrin, and Zn-porphyrin complex, the results depend strongly on the rigidity/viscosity of the environment. In Triton X-100 or an agar matrix, the species with lifetime 100 and 460 ns were observed, whereas only 100 ps lifetime was observed in organic media, such as toluene, THF, and benzonitrile. For another example of charge transfer in dendronized metalloporphyrin C_{60} conjugates, see Spanig et al. (2009).

Photofunctional nanomaterials composed of multiporphyrins and carbon-based π-electron acceptors were investigated as artificial photosynthetic systems (Fukuzumi and Kojima 2008).

Dendron-coated tetraphenylporphyrins and their Zn(II) complexes were studied by combination of several spectroscopies with DFT theoretical simulation of their infrared, Raman and electronic absorption spectra, and fluorescence emission (Minaev and Lindgren 2009). According to time-dependent DFT calculations, increased Jahn–Teller distortion of the porphyrin core for larger dendrimer generations was observed.

Dendrimers with porphyrin in the core, branches, or both were reviewed as potential nanodevices, light-harvesting systems (Jianga and Aida 2005), and catalysts (Liang and Frechet 2005; Martos et al. 2008; Rosen et al. 2009).

For other examples of porphyrin core-based dendrimers, see Drouet and Paul-Roth (2009), Li and Aida (2009), Maes and Dehaen (2009), Lebedev et al. (2009), Shema-Mizrachi et al. (2009), Iehl et al. (2009), Kimura et al. (2009), Albrecht et al. (2009), Chabre and Roy (2008) and for dendrimers with porphyrin located at the end of dendrimer branches, see Hasobe (2010), Li and Aida (2009), Maes and Dehaen (2009), Yang and Kim (2009).

There are many other types of dendrimers with the cyclic core, such as cucurbit[*n*]urils (Wang and Kaifer 2009), resorcarenes (Mendrek and Trzebicka 2009), cavitands (Freeman et al. 2009; Huttunen-Hennelly and Sherman 2008; Nikan and Sherman 2008, 2009), polyhedral

oligomeric silsesquioxanes (Ge et al. 2009), phthalocyanines (Li and Aida 2009; Chen et al. 2009a; Lebedev et al. 2009; Nishiyama et al. 2009), etc. (Rosen et al. 2009).

Dendrimeric libraries

In general, low affinity is obtained for interactions between carbohydrate receptors and modified oligosaccharides designed as mimetics of natural carbohydrate ligands. Therefore, glycopeptides have been explored as alternative mimetics. Glycopeptides in general have proven to be superior ligands with higher affinity for a receptor than the natural carbohydrate ligand (Niederhafner et al. 2008b). Two of the ways for creation and selection of new leads are glycopeptide and oligosaccharide libraries. This topic has been reviewed (Meldal et al. 2010; Darbre and Reymond 2006, 2008; Hojo and Nakahara 2007; Shin et al. 2005; Niederhafner et al. 2008b; Banik and Brady 2008; Murase et al. 2009; Maljaars et al. 2008; Renaudet 2008; Roy 2003; Roy and Touaibia 2007; Rosen et al. 2009) with different glycotopes (T_N, TF, mannose, GlcNAc, L-fucose, etc.), resins (TentaGel, PEGA, POEPOP, SPOCC, etc.), and applications. The libraries are accessible by synthesis in solution, on the solid phase, chemoenzymatically and in cell syntheses (Meldal et al. 2010; Niederhafner et al. 2008b; Banik and Brady 2008; Murase et al. 2009; Maljaars et al. 2008). This powerful technique can be used generally for the identification and analysis of complex interactions between carbohydrates and their receptors (Niederhafner et al. 2008b).

A library approach was applied for selection of inhibitors preventing biofilm formation of *P. aeruginosa* (Kolomiets et al. 2007; Johansson et al. 2007, 2008; Darbre and Reymond 2008; Kolomiets et al. 2009), which is a human pathogenic bacterium, producing a L-fucose-specific lectin, LecB, implicated in tissue attachment and the formation of biofilms. A 15 625-membered glycopeptide dendrimer combinatorial library (Kolomiets et al. 2007) with α-*C*-L-fucosyl residues at the N-termini was prepared on TentaGel and screened for binding to L-fucose-specific lectins. Glycopeptide dendrimer (L-Fuc-α-CH_2-CO-Lys-Pro-Leu)$_4$-(Lys-Phe-Lys-Ile)$_2$-Lys-His-Ile-NH_2 was identified as a potent ligand against *Ulex europaeus* lectin UEA-I (IC_{50} = 11 μM) and *P. aeruginosa* lectin PA-IIL (IC_{50} = 0.14 μM). Later, larger (390 625-member) self-encoded glycopeptide dendrimer library was screened (Johansson et al. 2007). The dendrimer disrupts established biofilms in the wild-type strain of *P. aeruginosa* and in several clinical isolates (Johansson et al. 2008). Further optimization of the structure with the aim to obtain inhibitors of biofilm formation in the *P. aeruginosa* pathogen led to inhibitors (L-Fuc-α-p-O-C_6H_4-CO-Lys-Ala-Asp)$_4$-(Lys-Ser-Gly-Ala)$_2$-Lys-His-Ile-NH_2 (Johansson et al. 2008) and (L-Fuc-α-CH_2-CO-Lys-Pro)$_8$-(Lys-Leu-Phe)$_4$-(Lys-Lys-Ile)$_2$-Lys-His-Ile-NH_2 (Kolomiets et al. 2009) with IC_{50} 0.11 and 0.025 μM, respectively. The last mentioned compound possessed 440-fold enhancement in potency over L-fucose (Kolomiets et al. 2009). The fucosylated glycopeptide dendrimers reported here seem particularly well suited to the development of polyvalent inhibitors of *P. aeruginosa* adhesion and biofilm formation (Fig. 13).

For the identification of α-galactosyl epitope mimetics TentaGel S NH_2 bound *C*-linked glycopeptide libraries have been used (Xian et al. 2004). 2,3,4,6-Tetra-*O*-acetyl-α-galactopyranosyl-(2-acetic acid) was used as a *C*-glycoside galactose building block. One-bead-one-compound library was screened with human anti-Gal Abs (IgG, IgM, and IgA), and finally MALDI sequencing served for identification of the mimetics of α-Gal epitopes. The found

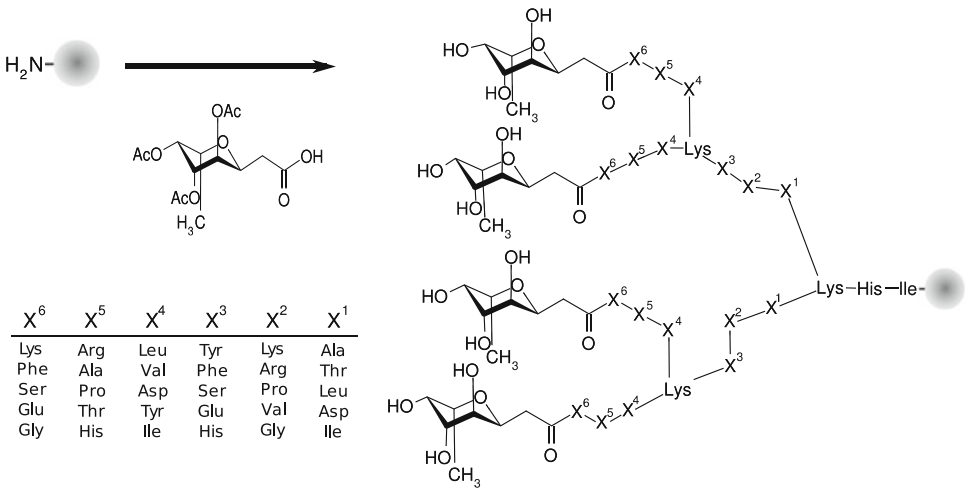

Fig. 13 Synthesis of the *C*-fucosyl peptide dendrimer library by split-and-mix SPPS on TentaGel resin (Kolomiets et al. 2009)

glycopeptides show better inhibition activities than those of known Gal-α-(1 → 3)-Gal peptide mimetics.

Click chemistry assisted by microwaves, starting from different poly alkyne DNA-based scaffolds and two galactosyl azide derivatives (Pourceau et al. 2008) was used for synthesis of small libraries of di-, tri-, and tetragalactosyl clusters on solid support. Standard DNA solid-supported phosphoramidite chemistry using a novel alkyne phosphoramidite and an alkyne solid support was used for the scaffold synthesis. The libraries were obtained with high purity suitable for direct biological evaluations. Besides, each family could be isolated by HPLC before evaluation. The synthesis of small libraries of di-, tri-, and tetragalactosyl clusters with a phosphodiester linkage containing 4, 8, and 16 compounds, respectively, using Cu(I) catalyzed azide–alkyne cycloaddition, proceeded smoothly.

Peptide and glycopeptide libraries prepared by the 1,3-dipolar cycloaddition between azides and alkynes have been reviewed (Tron et al. 2008), for details see "Click chemistry".

TASP was selected as topological cyclopeptide scaffold (Dulery et al. 2008). Oxime-based ligation strategy was used to secure a quantitative assembly of biomolecules with various functionalities. Imine or hydrazone linkages are useful for generating combinatorial libraries under thermodynamic control. Since the oxime bond is hardly reversible under ambient conditions, it was assumed that the generation of libraries from TASP and a mixture of several aminooxy building blocks should theoretically lead to the randomized and statistical distribution of each expected library species in a comparable amount. As a proof of concept for this randomized, mixture-based combinatorial library, the authors (Dulery et al. 2008) have done screening with a model lectin ConA. The binding of seven mixtures of five mannose-based hGC with ConA bound to an agarose gel was studied. The ligands were selected and shown by SPR that tyrosine influences the binding. This approach can be applied in glycomic or proteomic research, for discovery of glycomimetics or selective ligands.

New, important types of libraries are dynamic combinatorial libraries (DCL) (Macmillan and Daines 2003). The main goal for the synthesis of combinatorial libraries is the discovery of new drug leads. The production of DCL (Niederhafner et al. 2008b; Nestler 2005; Huc and Nguyen 2001; Roy 2003; Meyer et al. 2007) in the presence of a target should create a library of compounds skewed toward structures that interact favorably with that target. These reactions involved in such library formation must be reversible. DCL can be viewed as a temporary pool of compounds that are reversible assemblies of a mixture of building blocks. In the final mixture, the thermodynamically more stable product is amplified in concentration by equilibrium driven selection process (Macmillan and Daines 2003). The equilibrium is shifted by the presence of a template, to which the library members bind. Tight binders are preferred instead of those with weak binding or no binding ability. Structures identified from these libraries should be good hosts or guests for the introduced template, and therefore potential drug leads.

Self-replicating systems may have played an important role in the origin of life. DCL with dynamic cyclic core formed from 3,5-disulfanylbenzoic acid conjugated with several peptides was reported (Carnall et al. 2010). Replication of covalent structure from these conjugates was driven by nanostructure formation, based on the assembly of the peptides into fibers, which were held together by β sheets. Mechanical forces were used as selection factor for final covalent structure. Which of the two desired products became dominant was influenced by whether the sample was shaken or stirred. This is the first example of influence of mechanical forces on the outcome of a covalent synthesis.

Mass spectral quantities and distributions of the three DCLs of mechanically interlocked dendrimers ([4]rotaxanes, molecular weight up to 8 800 kDa), which were previously synthesized (Leung et al. 2007), have been theoretically evaluated (Leung 2009). The theoretical postulations based on dendrimer macroconstants in a combination with statistical distribution, binding constant, steric, and hydrophobic effects were used for an explanation of ESI mass spectral intensities. In a competitive mixture, these intensities are not random. The predictions of normalized MS intensities had good agreement with the experimental results for all dynamic dendrimers except for those with higher molecular weights. Using this theoretical postulation, prediction of the MS intensities of individual dendrimers was achieved in spite of having the same molecular weight (e.g. G0/G0/G2 and G1/G1/G1, etc.) and overlapping of their experimental MS signals.

To increase the diversity of libraries many building blocks, both natural and unnatural, have been prepared. Sugar amino acids (Maljaars et al. 2005; Gruner et al. 2002; Chakraborty et al. 2004, 2005, 2008; Jensen and Brask 2005; Simone et al. 2008) (glycosamino acids) represent an important class of polyfunctional scaffolds, in which the carboxyl, amino, and hydroxyl termini provide an excellent opportunity to create structural diversities akin to nature's molecular arsenal. Kessler (Gruner et al. 2002) defines sugar (carbohydrate) amino acid (SAA) as a compound with immediate linkages of both amino and carboxy functionalities to a carbohydrate frame. SAAs are broadly distributed in nature, with sialic acid and *N*-acetylmuramic acid being the most prominent examples.

A library of carriers based on various combinations of the cell penetrating peptide TAT, the SV40 Large T protein nuclear localization signal (NLS) and a cationic dendrimeric peptide was prepared and tested for its ability to deliver exogenous DNA to human HeLa cells (Yang et al. 2009c). The most active carrier consists of the TAT, NLS, and the dendrimeric peptides. Chloroquine was used as a DNA protective agent, which enhanced transcription of DNA bound to the respective carriers.

Heptapeptides selected from phage display, which bound to *Bacillus subtilis* spores, were used for construction of MAP scaffold (Lusvarghi et al. 2009). They were used as a probe for a flow cytometric assay. The tetravalent MAP scaffold enhanced the affinity for *B. subtilis* spores by more than 1 and 2 orders of magnitude when compared to divalent and monovalent analogs, respectively. These MAPs are effective most probably because they mimic the multivalent display of the original peptide library on the phage coat.

Detailed protocol of the synthesis, functional screening, and decoding of "one-bead-one-compound" combinatorial libraries of dendrimers assembled from amino-acid building blocks by "split-and-mix" SPPS was described (Maillard et al. 2009a). A branching diamino acid is attached at every third position to obtain the dendritic structure. Library of approximately 60,000 sequences was obtained. Due to restricted use of only four amino acids for splitting step, the sequence of a dendrimer can be obtained uniquely from an amino-acid analysis of the solid support bead. In comparison with Edman sequencing, this analysis is more reliable, faster, and far less costly. A search for catalysts of the hydrolysis of acyloxypyrene-trisulfonates was used as an example.

A highly efficient synthetic route toward bifunctional polyglycerol dendrons on a multigram scale was reported using click chemistry (Wyszogrodzka and Haag 2008). Williamson ether synthesis was combined with an ozonolysis/reduction procedure resulting in the fourth generation of glycerol-based dendrons. These dendrons have a reactive core, which was converted to the corresponding monoazido derivatives. Click chemistry was used for construction of a library of core–shell architectures.

Dynamically formed glycopolymers have been generated using reversible acylhydrazone ligation (Ruff et al. 2010). The molecular weight and the rate of exchange were tuned by selection of appropriate dialdehyde monomers. Dihydrazide monomers and aromatic dialdehydes formed high molecular weight glycopolymers showing reversibility under harsh conditions, but stability under physiological conditions. Due to their inherent fluorescence, these glycopolymers may serve as biosensors of lectins, bacteria, toxins, viruses, etc. Their glyco parts make them suitable for lectin binding.

For other examples of dendrimer libraries, see Chabre and Roy (2010), Percec et al. (2009), Imam et al. (2009).

Dendrimers as catalysts

Dendrimer-encapsulated nanoparticles are used as catalysts both in homogeneous and in heterogeneous catalysis (Astruc et al. 2010; Peng et al. 2008; Svenson 2007; Scholl et al. 2009; Helms and Frechet 2006; Liang and Frechet 2005; van Dongen et al. 2009). Dendrimers are attractive for catalytic applications, because they can act as catalytically active species, as well as soluble supports onto which catalytically active species can be attached (Scholl et al. 2009).

Lo and Chow (2009) described synthesis of chiral amphiphilic G1–G3 dendritic organocatalysts possessing an optically active polar proline-derived core and one or two non-polar hydrocarbon dendrons. These dendritic organocatalysts were used in the asymmetric aldol and nitro-Michael additions in oil-in-water emulsions to study the effects of dendron size and branching on the catalytic properties. The incorporation of larger hydrophobic dendrons promotes emulsion formation in water, improves the reaction enantioselectivity, decreases catalyst loading (to 1 mol%), and facilitates catalyst recovery after the reactions. Due to increasing steric blocking effect of the larger dendrons, lower catalytic activity was observed. Surprisingly, in some of the G1 and G2 dendritic organocatalysts an increase in the steric bulkiness and branching of the dendron resulted in better catalyst reactivity. Another advantage is reusability of the dendritic catalyst up to five times without significant drop in product yields and enantioselectivity. Moreover, cross contamination was not observed when the recovered G3 catalyst was subsequently used in another reaction involving different substrates.

Enantioselectivity in a catalytic or stoichiometric reaction is governed by small increments of free enthalpy of activation. Such transformations are thus in principle suited to assessing "dendrimer effects" which result from the immobilization of molecular catalysts. Chiral dendrimer catalysts with a high level of molecular monodispersity, structural regularity, and well-defined catalytic sites, have been prepared either by immobilization of chiral catalysts to achiral dendrimers or by attachment of achiral complexes to chiral dendrimer structures. The topic of stereoselective dendrimer catalysis was reviewed (Kassube and Gade 2006).

Nanodevices with Buckminsterfullerene (C_{60}) shell bound to a G4 PAMAM core, containing approximately 30 shell fullerenes on each dendrimer core (Jensen et al. 2005) were used to catalyze photooxidation of thioanisole by generation of singlet oxygen ($^1O_2$). The reactivity is

enhanced in aqueous solution, possibly due to a nanoreactor effect resulting from diffusion of hydrophobic reactant molecules into dendrimer cavities.

Phosphorous glycodendrimers have been prepared (Hadad et al. 2009) in high yields by grafting xylose-derived moieties on phosphorous dendrimers using hydrazone units. These dendrimers can be amphiphilic or hydrophobic, respectively, with unprotected or acetylated sugar moieties. These compounds were studied as potential candidates for asymmetric or micellar catalysis.

Carbo-BINAP ligands have been immobilized on PPI dendrimers as soluble supports (Kassube et al. 2009). They contained up to 64 BINAP ligands per molecule. The immobilization strategy has been extended to less regularly hyperbranched PEI as soluble supports. It was possible to attach on average 9, 26, and 138 Glutaroyl-AMINAP or Carbo-BINAP ligands to PEIs of different molecular weights. Their catalytic properties were studied on the copper-catalyzed hydrosilylation of acetophenone. The PPI-bound Carbo-BINAP dendrimers displayed a strong dependence of enantioselectivity and activity on the generation. It is interesting that the macromolecular, immobilized BINAP ligands could be recycled several times without any observable loss of activity or enantioselectivity.

Cyclodextrins, calixarenes, and dendrimers-based metal complexes were used as catalysts for hydroformylation, Wacker oxidation, hydroxylation of aromatics, 2-naphthol coupling, and oxidative coupling of styrenes and benzene (Karakhanov et al. 2008).

An important goal in catalysis is to unite the advantages of homogeneous and heterogeneous catalytic approaches. Nanoparticles play crucial role in heterogeneous catalysis providing new or divergent reactivity and selectivity. Toste et al. (Witham et al. 2010) reported a new approach for applying heterogeneous catalysts to homogeneous catalytic reactions by the use of electrophilic platinum nanoparticles. They are selectively oxidized by the hypervalent iodine species $PhICl_2$, and catalyze a range of π-bond activation reactions, which were previously catalyzed only through homogeneous processes. Modification of nanoparticles leading to the desired homogeneous catalytic activity can serve for further development of reactions previously inaccessible in heterogeneous catalysis.

A photocleavable library of up to 65,536 peptide dendrimers $(AcX^8X^7)_8(DapX^6X^5)_4(DapX^4X^3)_2DapX^2X^1$ (Dap = (S)-2,3-diaminopropionic acid branching point, X^{8-1} = groups of four proteinogenic amino acids) was synthesized on TentaGel resin (Maillard et al. 2009b). The library was tested as hydrolytic catalyst of the fluorogenic substrate 1-butyryloxy-pyrene-2,7,8-trisulfonate and its derivatives. Histidine catalytic activity at the dendrimer core was suppressed by anionic glutamate residues in the outer dendrimer branches. The simplicity of implementation of the "off-bead" in silica assay allows its transfer for other library and reaction types.

For more details about use of dendrons and dendrimers in catalysis, see Liang and Frechet (2005), Kehat et al. (2007), Lo and Chow (2009), Svenson (2007), Scholl et al. (2009), Kassube and Gade (2006), Helms and Frechet (2006), Dahan and Portnoy (2005), Niederhafner et al. (2008b), Maillard et al. (2009b), van Dongen et al. (2009).

Dendrimers and solubility

Poor solubility and hydrophobicity of drugs and other bioactive compounds are limiting factors of their possible applications in drug delivery and formulation development. About 40% of newly developed drugs are rejected by the pharmaceutical industry and will never benefit a patient because of poor bioavailability due to low water solubility and/or cell membrane permeability (Astruc et al. 2010; Gupta et al. 2006; Svenson and Chauhan 2008; Svenson 2009; Svenson and Tomalia 2006; Shi et al. 2009). For the same reason another 17% of drugs exhibit low performance. Due to these facts, a deep understanding of enhancement of drug solubility and bioavailability is a crucial task for drug delivery. Dendrimers constitute a novel type of material with unique structure and properties which are suitable for drug solubilization. Solubilizing properties of dendrimers are attributed to ionic interactions, hydrogen bonding, and hydrophobic interactions. Dendrimers and micelles have hydrophobic interior capable of encapsulating hydrophobic drugs and they possess hydrophilic exterior responsible for solubilization (Gupta et al. 2006; Svenson and Chauhan 2008; Svenson 2009; Shi et al. 2009). Drugs can be solubilized by dendrimers by simple encapsulation (**7**, **8**), covalent conjugation (**9**, **10**, **11**, **13**) or electrostatic interaction (**12**, **13**) (Fig. 14) (Rolland et al. 2009; Cheng et al. 2007, 2008a, b; Cheng and Xu 2008; Tong and Cheng 2007; Patri et al. 2005; Tekade et al. 2009b; Rosen et al. 2009). Dendrimer-based drug delivery improves the drug bioavailability and moreover, it provides protection of drug molecules against enzymatic or hydrolytic breakdown in the body (Svenson and Chauhan 2008; Svenson 2009; Tekade et al. 2009b). Effect of generation, size, pH, core, branches, temperature, polymeric architecture, and end groups and their modifications (charge, hydrophilicity vs. hydrophobicity) on the solubility enhancement of dendrimers were studied by many authors (Gupta et al. 2006; Chadha et al. 2008; Svenson and Chauhan 2008; Svenson 2009; Svenson and Tomalia 2006; Bai et al. 2006; Cheng et al. 2007, 2008b; Rolland et al. 2009; Paleos et al. 2007, 2008; Balogh 2007; Dufes et al. 2005; Patri et al. 2005; Tekade et al. 2009b; Rosen et al. 2009).

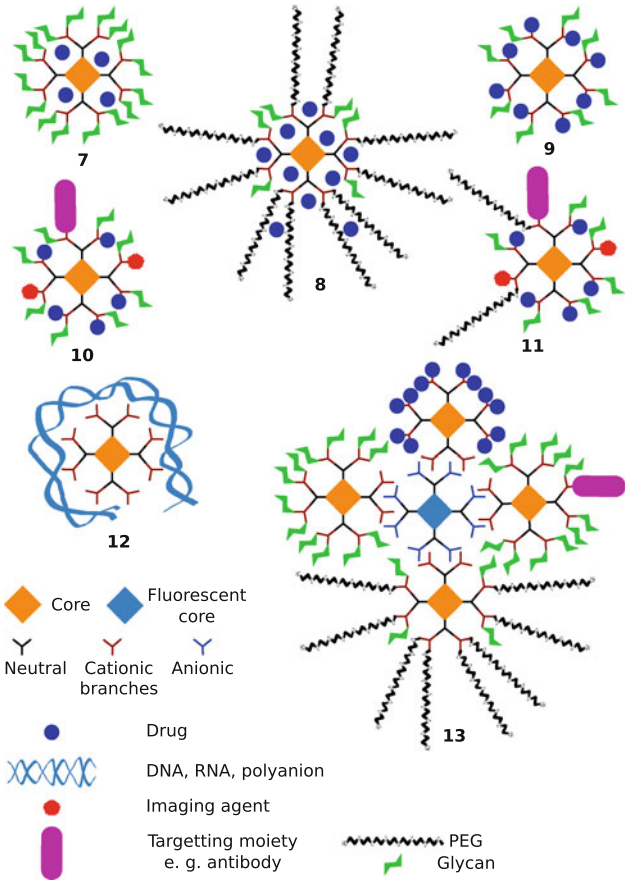

Fig. 14 Various ways of drug solubilization by dendrimers (Tekade et al. 2009b)

Dendrimers were used as solubility enhancers for many drugs, such as anticancer drugs (camptothecin, carboplatin, chlorambucil, cisplatin, dimethoxycurcumin, doxorubicin, etoposide, 5-fluorouracil, methotrexate, oxaliplatin, and paclitaxel), antidepressants (venlafaxine), antifungal drugs (amphotericin B), antihemorrhagic drugs (nimodipine), antihistaminics (famotidine), anti-inflammatory drugs (diclofenac, diflunisal, ibuprofen, indomethacin, ketoprofen, mefenamic acid, methylprednisolone, naproxen, nifedipine, phenylbutazone, and piroxicam), and antimicrobial drugs (artemether, niclosamide, nadifloxacin, penicillin V, prulifloxacin, and sulfamethoxazole). This topic was covered be excellent reviews (Svenson and Chauhan 2008; Svenson 2009; Svenson and Tomalia 2006; Tekade et al. 2009b; Villalonga-Barber et al. 2008; Prajapati et al. 2009; Gupta et al. 2007; Zhou et al. 2009; Daia et al. 2009; Ouyang et al. 2009; Zhao et al. 2009; Yellepeddi et al. 2009).

Another way to achieve solubilization and controlled release of chemotherapeutics is the use of PEGylated dendrimers (Tekade et al. 2009b).

Fullerene C_{60} represents a unique structure with notable chemical and physical properties, and was studied in diverse fields including biological applications. The limiting parameter is extremely poor solubility of fullerenes in water which hampers their usage for biomedical applications (Kojima et al. 2008). PAMAM dendrimers having both β-CD and PEG were synthesized and characterized by ^{1}H NMR, IR, and gel permeation chromatography. The achieved C_{60} concentration in the aqueous solution of the PEG_{46}/CD_{18}-modified dendrimer (2.0 μM) was 2.8 μM, although the solubility of C_{60} in pure water is reported as 2×10^{-24} M. This represents 10^{18} increase of C_{60} solubility in water. This was attributed to the clustering effect of CD and PEG at the surface of the dendrimer (Fig. 15).

PAMAM-b-poly(L-glutamate) (PAMAM-b-PLG) biohybrids were synthesized by the ring-opening polymerization (Qiu et al. 2009). The critical aggregation concentration of PAMAM-b-PLG is influenced by pH. The self-assembled nanoparticle size decreased with the increasing pH value of its solution. The self-assembled nanoparticles had a nearly spherical morphology. The pH-influence on the self-assembly behavior was attributed to changes from α-helical to random coil conformation.

For more details about the influence of dendrimers on solubility, see Gillies and Frechet (2005), Svenson and Tomalia (2006), Balogh (2007), Cheng et al. (2007), Svenson and Chauhan (2008), Cheng et al. (2008b), Bai et al. (2006), Svenson (2009), Paleos et al. (2007), Tekade et al. (2009b), Gupta et al. (2006). This topic is closely related to "Dendrimers in drug delivery".

Biocompatibility and toxicity of dendrimers

Biocompatibility (or toxicity) of dendrimers has been reviewed (Astruc et al. 2010; van Dongen et al. 2009; Yellepeddi et al. 2009; Rolland et al. 2009; Svenson and Chauhan 2008; Cheng et al. 2007, 2008b; Svenson 2009; Boas et al. 2006b; Patri et al. 2005; Labieniec and Watala 2009; Heegaard et al. 2010; Biricova and Laznickova 2009; Dufes et al. 2005; Svenson and Tomalia 2005; Lee et al. 2005a; Gillies and Frechet 2005; Duncan and Izzo 2005; Niederhafner et al. 2005, 2008b; Boas and Heegaard 2004; Heegaard and Boas 2006; Tsvetkov and Nifantiev 2005; Bezouska 2002; Yang and Kao 2006; Svenson and Tomalia 2006; Crampton and Simanek 2007; Svenson 2007; Muthu and Singh 2009; Paleos et al. 2007, 2009). Biomedical applications require low toxic and non-immunogenic dendrimers. The term "toxicity" is often applied in pharmaceutical industry for description of unwanted side effects to cells, organs, or the patient. The term "biocompatibility" is used in the field of biomedical materials and their applications as a measure of their compatibility with the given organ or organism. The more compatible, the less toxic it is and vice versa. At a consensus conference of the European

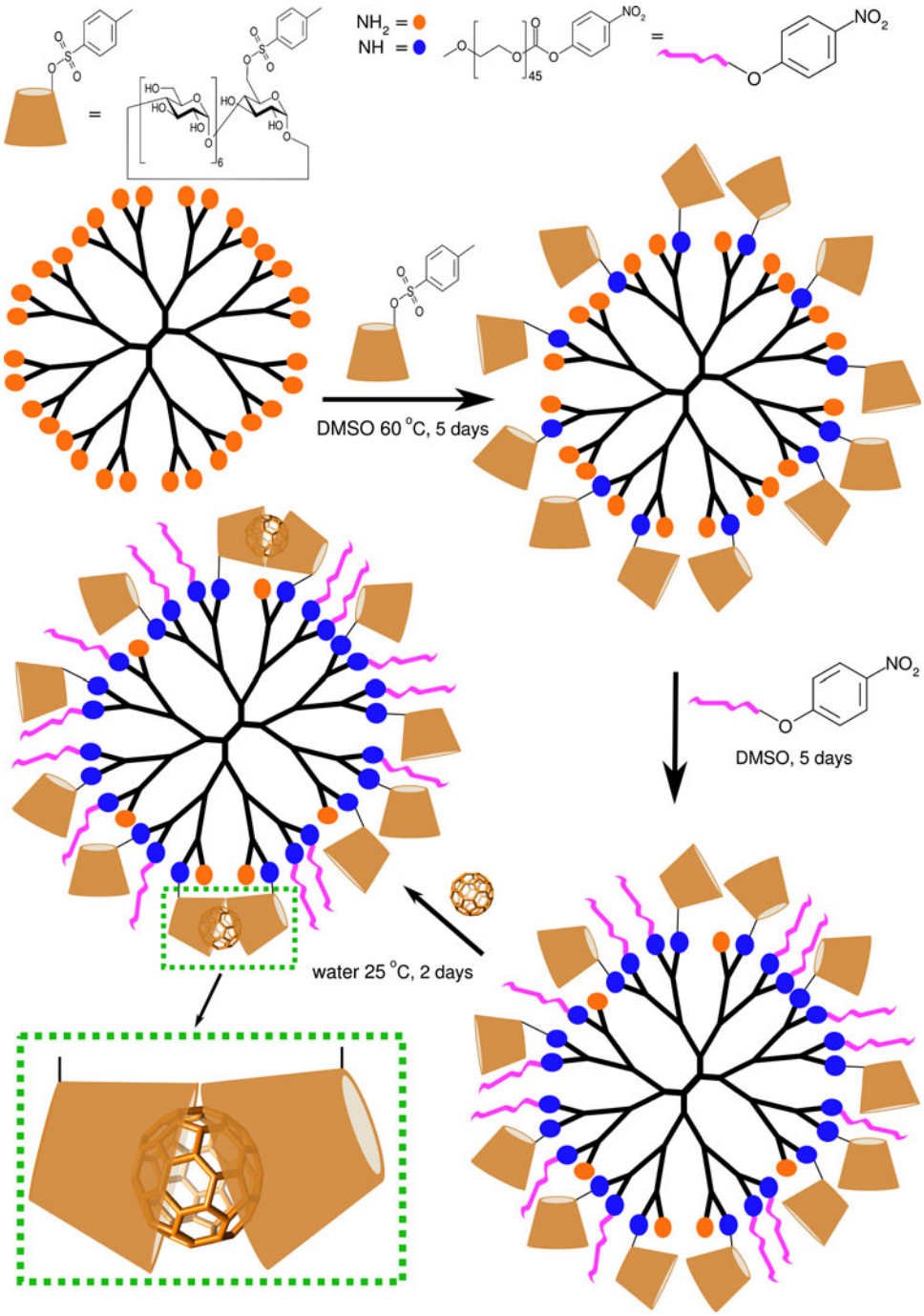

Fig. 15 Synthesis of PAMAM dendrimer containing both β-CD and PEG and their influence on amplified solubility of C_{60} fullerene by 18 orders of magnitude (Kojima et al. 2008)

Society for Biomaterials in 1986, biocompatibility was defined as the capability of a material to act with a proper host response in a specific application (Duncan and Izzo 2005; Williams 1989; Niederhafner et al. 2008b). Hence, it is necessary to define a material "biocompatibility" only if the precise context of its use is stressed. Without detailed knowledge of the intended precise use, it is not possible to define any dendrimer chemistry as non-toxic or biocompatible. Due to limited clinical experience with dendrimers, it is impossible to designate any particular chemistry intrinsically "safe" or "toxic" (Duncan and Izzo 2005).

Amino surface groups of dendrimers significantly influence biological membranes due to electrostatic interactions of their high positive charge and the negative charge of most cell membranes (Heegaard et al. 2010; Niederhafner et al. 2008b; Tomalia et al. 2007; Gajbhiye et al. 2007). This results in a high cellular uptake of these molecules by endocytosis. Higher-generation of amino terminated

dendrimers (more than G3) caused more destructive interaction with the membrane, which led to cellular lysis and high cytotoxicity. On the other hand, negatively charged dendrimers (carboxylic acid surfaces) do not interact with most cellular membranes and therefore do not have generation-dependent cytotoxicity. When non-charged dendrimers are studied, their cytotoxicity depends on polarity of surface groups. Dendrimers with polar membrane non-invasive groups like PEG have a non-toxic behavior. On the contrary, non-polar groups like lipids interact with the cellular membrane by hydrophobic interactions. This renders in some cases the dendrimer cytotoxic. Lipids can also have positive influence because they can endow dendrimers with immunostimulating properties. Biodistribution, clearance, and toxicity are strongly dependent on the particle size. Nanoparticles in general, including dendrimers and their toxicity with respect to particle size have been reviewed, see "Dendrimers in nanoscience and nanotechnology".

Clinical applications of PAMAM dendrimers in drug delivery are limited due to their inherent cytotoxicity, reticuloendothelial system uptake, and hemolysis (Yellepeddi et al. 2009). The toxicological response of PAMAM dendrimers correlated with the dendrimer generation (Naha et al. 2009; Mishra et al. 2009). The higher the particle surface area the higher is the toxic response. Their toxicity could be significantly reduced by surface modifications. PEG conjugation significantly decreased the in vitro and in vivo cytotoxicities and hemolysis of G5 and G6 PAMAM dendrimers (Qi et al. 2009). Zebrafish can serve as a correlative and predictive model for assessing biomaterial nanotoxicity (Fako and Furgeson 2009).

To address a common pitfall of leukemia therapy—the development of drug resistance—PAMAM dendrimers were used for a dual drug delivery of antileukemic drugs (Tekade et al. 2009a). One molecule of PAMAM dendrimer was found to be capable under optimized conditions of pH and dialysis time to entrap approx. 27 and 8 molecules of methotrexate and all-trans retinoic acid, respectively. The release kinetics depends on the degree of dendrimer protonation, with more sustained and controlled behavior at pH 7.4. A cytotoxicity study on HeLa cell lines has proven that dual drug loaded dendrimer was more efficient than the free drug combination.

Cytotoxicity, hemolysis, genotoxic effects, interactions with model lipid membranes, and other biological properties of low-molecular mass lysine-based peptide dendrimers with antibacterial activity have been studied (Klajnert et al. 2006a, b). The dendrimers with protected ε-amino groups were the most toxic in all tests, whereas only low toxicity was observed for those with protected α-amino groups. Both toxicity and antimicrobial activity are influenced by the steric distribution and type of hydrophobic groups and cationic centers.

For other papers dealing with dendrimer toxicity and biocompatibility, see also Wang et al. (2010b).

Dendrimers in nanoscience and nanotechnology

In ancient Greek "Nano" means dwarf. One nanometer is one-billionth of a meter, 10^{-9} m. This is roughly four times the diameter of an individual atom. Width of DNA is approximately 2.5 nm and protein molecules measure 1–20 nm. Since nanotechnologies are the design, production, characterization, and application of structures, devices and systems for controlling size and shape at nanometer scale, they can manipulate and create materials at the atomic scale (Stylios et al. 2005; Jain 2008b).

Nanotechnology creates useful materials, devices, and systems through the manipulation of tiny matter (including anything with at least one dimension less than 100 nm). The interdisciplinary field of nanotechnology involves physics, chemistry, biology, medicine, etc. Nanoscale devices are about 100–10,000 times smaller than human cells and are similar in size to large biological molecules, such as enzymes and receptors, as shown in Fig. 16 (Stylios et al. 2005). Their diameters are in range of 1–100 nm, their molecular masses are within interval 10^4–10^7 Da and they are usually built up from 10^3–10^9 atoms (Tomalia

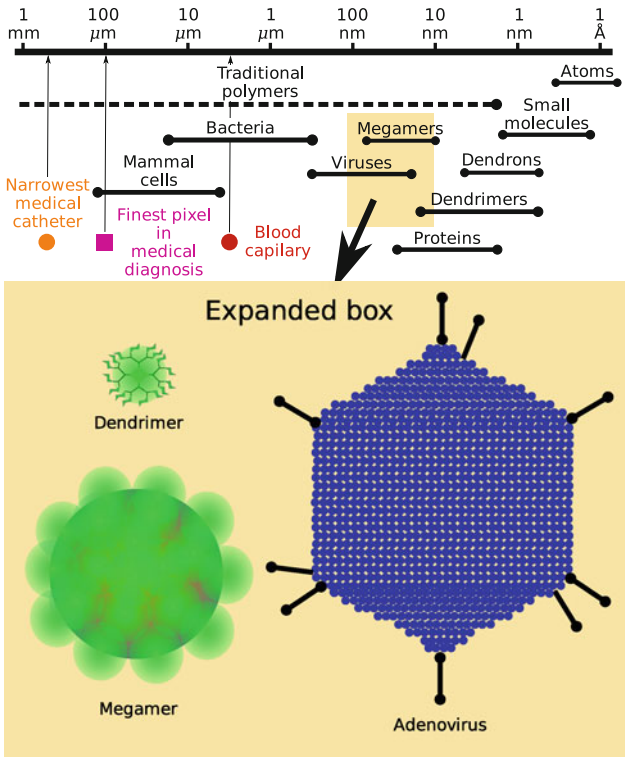

Fig. 16 Comparison of nano-, meso-, micro-, and macro-objects (Stylios et al. 2005)

2009). Therefore, nanoscale systems have the ability to interact on both the surface and the inside of cells. This allows them to detect diseases and deliver the drug cargo to the right place of the body in a way unknown so far. The tailor made artificial nanostructures can sense and repair damaged parts of our body. In this way they can act like naturally occurring biological nanostructures, e.g. the white blood cells (Stylios et al. 2005). Nanotechnology has many similar features and overlaps with another multidisciplinary field of dendrimers (Tomalia 2005b, 2009; Martini and Ciani 2009; Yellepeddi et al. 2009; Tomalia and Majoros 2003; Stylios et al. 2005; Svenson and Tomalia 2006; Jain 2008b; Smith et al. 2005; Svenson 2009; Rolland et al. 2009; Svenson and Chauhan 2008; Bharali et al. 2009; Portney and Ozkan 2006; LaRocque et al. 2009; Jensen et al. 2005; Dutta et al. 2010; Rosen et al. 2009; Sekowski et al. 2008; Samad et al. 2009; Muthu and Singh 2009; Chabre and Roy 2010).

Liposomes have been used as potential carriers with unique properties, e.g. protecting drugs from degradation, targeting to site of action, and reduction of toxicity and other side effects. Their practical applications are limited due to inherent problems, such as poor storage stability, low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components, etc. In comparison with liposomes, nanoparticles (Smith et al. 2005) offer some specific advantages. Nanoparticles help to increase the stability of drugs/proteins and possess useful controlled release properties as delivery systems (Jain 2008b).

Drug-loaded dendrimers can be used as nanovehicles (Tekade et al. 2009b; Rolland et al. 2009).

There are many sorts of materials, technologies, and applications with the prefix nano: nanoarrays, nanobalances, nanobelts, nanobiotechnology, nanobridges, nanocatalysts, nanochannels, nanoclusters, nanocomposites, nanocrystalline materials, nanocrystals, nanocubes, nanodiamonds, nanodisks, nanodots, nanoelectrodes, nanoelectromechanical systems, nanoelectronics, nanoenvironments, nanofabrication, nanofibers, nanofingers, nanoislands, nanolenses, nanolithography, nanomanipulation, nanomaterials, nanomedicine, nanoparticle conjugates, nanoparticles, nanoparticle synthesis, nanopatterning, nanophotonics, nanopillars, nanoplates, nanopores, nanoprinting, nanoprisms, nanorings, nanorods, nanoscale manipulation, nanoscience, nanoshells, nanoslits, nanostructured materials, nanostructured surfaces, nanostructures, nanotechnology, nanotips, nanotoxicology, nanotube binding, nanotubes, nanowire arrays, nanowires, etc. (Tomalia 2005b, 2009; Fako and Furgeson 2009; Tomalia and Majoros 2003; Stylios et al. 2005; Svenson and Tomalia 2006; Jain 2008b; Smith et al. 2005; Svenson 2009; Rolland et al. 2009; Svenson and Chauhan 2008; Bharali et al. 2009; Portney and Ozkan 2006; Jensen et al. 2005; Dutta et al. 2010; Boturyn et al. 2008; Rosen et al. 2009; Sekowski et al. 2008; LaRocque et al. 2009; Samad et al. 2009; Aillon et al. 2009; Muthu and Singh 2009; Chabre and Roy 2010).

The set of nanomaterials partly overlaps with the set of dendrimers. The overlap can be physical (size), chemical, biological activity, etc. In the following text we give some examples, where dendrimers (especially glyco and glycopeptide dendrimers) are bound to the given type of nanostructure. These dendrimeric nanostructures have new quality of physical, chemical, and biological properties, e.g. solubility, stability, ability to work as delivery systems, and many others. The tremendous potential of nanotechnology contributes to prevention, detection, diagnosis, and treatment of cancer, viral, and bacterial diseases.

For reviews about the "nano" topic, see Rolland et al. (2009), Tekade et al. (2009b), Martini and Ciani (2009), Yellepeddi et al. (2009), Aillon et al. (2009), Larsen et al. (2006), Chadha et al. (2008), Tomalia (2005b), Tomalia and Majoros (2003), Frechet (2003), Frauenrath (2005), Smith (2006), Fernandez et al. (2006), Portney and Ozkan (2006), Mastrobattista et al. (2006), Smith et al. (2005), Shi et al. (2005b), Bhadra et al. (2005), Ribeiro et al. (2005), Khan et al. (2005), Svenson (2007), Peng et al. (2008), Scholl et al. (2009), Borm and Muller-Schulte (2006), Duncan and Izzo (2005), Paleos et al. (2007, 2009), Bai et al. (2006), Tong and Cheng (2007), Wolinsky and Grinstaff (2008), Svenson and Tomalia (2005), Schafmeister (2007), Cheng et al. (2008a), Tomalia et al. (2007), Carlmark et al. (2009), Tomalia (2005a), Emerich and Thanos (2008), Hamilton and Harth (2009), Cheng et al. (2008b), Bharali et al. (2009), Wu et al. (2008), Chen et al. (2006), Martin et al. (2009), Jianga and Aida (2005), Liang and Frechet (2005), Frauenrath and Jahnke (2008), Niederhafner et al. (2008a, b), Kobayashi and Brechbiel (2005), Tomalia (2009), Dutta et al. (2010), Lockman et al. (2005), Dufes et al. (2005), Jain (2008b), Boturyn et al. (2008), Rosen et al. (2009), Samad et al. (2009), Fako and Furgeson (2009), Chabre and Roy (2010).

The higher the generation of dendrimers the lower is the accessibility of the interior; the higher the increasing persistency of the globular shape the higher is the rigidity of the molecules. The accessibility of the surface is significantly greater than that of the interior-active species which will react first on the surface-exposed functions and they need some time to diffuse into the interior. Due to decreased flexibility and increased rigidity of the dendrimer as a function of generation, these higher generations of dendrimers cannot penetrate each other, and can interact solely on their surfaces; they behave as individual organic nanoparticles. These organic nanoparticles possessed perfect structural symmetry and extreme relative density (Balogh 2007).

Nanodevices can be made from dendrimers by covalent attachment of many different molecules and building blocks. A nanodevice was defined by Balogh as a polyfunctional macromolecule/nanoparticle that is able to perform different functions. By conjugation of dendrimers with various molecules, several functions of nanodevices are available, such as solubilization (PEGylation), regulation of charge (acetylation), light harvesting (conjugation with dyes), imaging (chelating ligands), drug delivery (targeting sugars, peptides or antibodies), etc. (Balogh 2007).

Nanomaterials have special properties—chemical, optical, magnetic, biological—which make them desirable for commercial or medical applications, such as cancer diagnosis (Kumar 2007) and drug delivery systems (Jain 2008a) which are easily tunable by type of material, shape, and size. However, these same properties can cause a response in the human body that is different from and is not directly predicted by the constituent chemicals and compounds. Even a traditional compound, such as carbon, can behave differently in the body when it is introduced as a nanomaterial. For example, carbon nanotubes can possess similar properties like asbestos (Kumar 2006; Aillon et al. 2009). Owing toxic effects of some nanomaterials, the world's scientists, industry, and governments are beginning to take a critical look at nanotechnology and to develop research methods for addressing key issues of the impact of nanotechnology on health and the environment. Due to the limited research data currently available about nanomaterial toxicity, new elements of screening strategy should be pinpointed rather than a detailed testing protocol. It is very probable that the biological activity of nanoparticles will depend on physicochemical parameters not routinely considered in toxicity assays. Thus, improper screening assays (Kumar 2006) can lead to false-negative results in toxicity screening.

To display mannose on the surfaces of iron oxide nanoparticles and polymer vesicles, they were coated with both dendritic and non-dendritic species (Martin et al. 2009) and their activities were determined by the hemagglutination assay. Dendritic functionalized vesicles and nanoparticles bound hemagglutinin with enhancement of 1–2 orders of magnitude in contrast to the non-dendritic system. It was caused by increased availability of the dendritic molecules on the surface due to their lower susceptibility to their burial within the polymer coating. Thus, this study reveals that the binding affinity of biological ligands presented at polymer surfaces can be significantly enhanced using dendritic scaffolds.

Carbon nanotubes (CNTs) have been widely explored in many biomedical applications. However, their inherent cytotoxicity is a limiting factor. The glycodendrimers based on 2,2-bis(hydroxymethyl)propionic acid, a biocompatible building block, were used as homogeneous bioactive coatings for CNTs (Wu et al. 2008) The dendrimers have a pyrene tail capable of binding single-walled carbon nanotubes (SWNTs) surfaces through π–π interactions and peripheral carbohydrate units. These glycodendrimers with a variety of carbohydrate structures were prepared by click chemistry in nearly quantitative yield. Adsorption of the glycodendrimers onto SWNTs was achieved by ultrasonication in aqueous solution, which resulted in formation of metastable colloid solutions. These solutions of glycodendrimer-functionalized SWNTs were stable for several months in water, whereas the non-functionalized SWNTs precipitated within 1 h. Sensing and targeting applications of dendrimers call for specific binding of SWNT-bound glycodendrimers to receptors. This capability was tested with a panel of (FITC)-conjugated lectins: ConA, *Arachis hypogaea* agglutinin (PNA), and *Psophocarpus tetragonolobus* agglutinin (PTA), which recognize α-mannose, lactose, and β-galactose, respectively. Significant fluorescence was found for ConA-treated with third generation of Man-SWNTs. Similarly, binding of third generation Gal-SWNTs to FITC-conjugated PTA were observed. Since the non-reducing terminal monosaccharide in Lac is Gal, third generation Lac-SWNTs were recognized by both PNA and PTA, but not ConA. Finally, third generations of glycodendrimer coating of SWNTs (100 μg mL^{-1}) completely suppress cytotoxicity of SWNTs toward HEK293 cells. Notably, the relatively thin coating of glycodendrimers appears to prevent SWNTs against cytotoxicity as effectively as much thicker glycopolymer coatings (Chen et al. 2006). Because glycodendrimer coating of SWNTs turns off SWNT cytotoxicity, it allows their applications in different receptor interactions, delivery of agents that target specific cell-surface receptors, biosensors for carbohydrate-binding proteins, etc. (Wu et al. 2008).

The same group (Chen et al. 2009b) reported that boron nitride nanotubes (BNNTs), which are isosteres of CNTs with unique physical properties, are inherently non-cytotoxic. Boron nitride has a stable hexagonal structure analogous to graphite. Besides their structural similarity, BNNTs and CNTs have similar thermal conductivity and mechanical properties. Almost the same glycodendrimers with various glycans as in the paper mentioned above (Wu et al. 2008) were used for coating of BNNTs. The G2 α-mannose-coated BNNTs were stable in aqueous solution for weeks, in comparison with the non-functionalized BNNTs, which precipitated within 1 h in water. Transmission electron microscopy (TEM) revealed high-purity and quality multiwalled BNNTs with an outer diameter of ~20–30 nm and a length of up to 10 mm. TEM images of the coated BNNTs have proven the presence of the glycodendrimers as an amorphous surface layer. Other glycodendrimers behaved similarly. It was proven, that

glycodendrimer-functionalized BNNTs can bind to proteins via ligand–receptor interactions, resisting non-specific binding of irrelevant proteins. The key finding is the ability of BNNTs to be surface functionalized with biological epitopes that mediate protein and cell binding. The authors (Chen et al. 2009b) have also shown that BNNTs can deliver DNA oligomers to the interior of cells with no apparent toxicity. This work suggests that in biomedical applications BNNTs may be superior to CNTs.

Gold glyconanoparticles (GNPs) imitating glycosphingolipids on the cell surface have been prepared (Rojo et al. 2004). They are non-toxic, stable against enzymatic degradation and highly soluble under physiological conditions. Because tumor-associated carbohydrate antigens are involved in the initial step of tumor spreading, a mouse melanoma model was used to test GNPs as possible inhibitors of experimental lung metastasis. Lactose-GNPs significantly reduce the progression of experimental metastasis. This supports the hypothesis that carbohydrate–carbohydrate interactions are the first step of the recognition process. This shows that biological effect of lactose-GNPs has potential application in biological processes. The anti-adhesive activity of GNPs as a tool in tumoral metastasis progression in vivo has been stressed.

β-CD-SAM formed on the glass slide and on the polystyrene beads were glued together by adamantyl-functionalized dendrimers (Ling et al. 2009) by non-covalent interactions. These supramolecular crystals are highly resistant to agitation by ultrasonication. They can be used as ink for a poly(dimethylsiloxane) (PDMS) stamp and can be transfer-printed onto a CD-functionalized target surface. The shape and size of the stamp can be varied leading to single particle lines, interconnected particle rings, and V-shaped particle assemblies. These particles play a role of 3D receptors capable to bind multiple complementary guest molecules. This shows that the supramolecular host functionalities of the particle crystals were retained throughout the fabrication process.

An important goal of nanotechnology, i.e. regenerable surfaces and reversible attachment of nanostructures onto them, was achieved by the adsorption and desorption of β-CD-functionalized nanoparticles onto and from stimuli-responsive preadsorbed ferrocenyl-functionalized PPI dendrimers at a β-CD SAM (Fig. 17) (Ling et al. 2008). Desorption of nanostructures from the β-CD SAMs was induced by electrochemical oxidation of the ferrocenyl endgroups. Nanoparticles remained robustly bound on the surface in the non-oxidized area. On the electrochemically oxidized area complete removal of nanoparticles was observed.

Molecular models of SAM were constructed using the gold (111) printboard and β-CD with both alkanethiol and alkanethioether chain linkers (Gannon et al. 2009). Coating of the surface with multivalent molecules was simulated with a divalently bound G0 ferrocene-terminated PAMAM dendrimer. Fully atomistic molecular dynamics (MD) computer simulations were used to probe the printboard lattice constant, height, steric packing, hydrophobicity, and ink-binding properties as a function of gold-β-CD "linker" molecule and the degree of binding to gold.

The same group described coarse-grained molecular dynamics simulations of nanopatterning with multivalent inks based on β-CD SAMs (Cieplak and Thompson 2008).

A series of functional nanotubes formed by cooperative self-assembly of dendrons and CD and their nanotube–nanoparticle hybrids were designed and used as "nanotube toolkit" (Park et al. 2008). These nanotubes play a role of protein sensors via specific binding of proteins on the tube surface. This toolkit has a tremendous potential for the construction of functional nanomaterials.

Dendrimers in drug delivery

The form in which drugs are delivered by dendrimers can be simple encapsulation, electrostatic interaction, or covalent conjugation (Astruc et al. 2010; Rolland et al. 2009; Cheng et al. 2007; Biricova and Laznickova 2009; Martini and Ciani 2009; Cheng and Xu 2008; Cheng et al. 2008a, b; Tong and Cheng 2007; Patri et al. 2005; Yellepeddi et al. 2009; Yoon and Jang 2010; Fox et al. 2009; Samad et al. 2009; Muthu and Singh 2009). The term "inclusion complex" is sometimes used to depict dendrimer containing a free drug bound by non-covalent interaction and the term "conjugate" was used to represent a drug covalently conjugated to dendrimer (Patri et al. 2005).

Limited clinical experience using dendrimers prevents a rational design of any particular system which is safe and non-toxic (Samad et al. 2009). However, there are several examples of promising systems for drug delivery.

Dendrimer–drug conjugates can be applied by oral, intravenous, intraperitoneal, intratumoral, transdermal, ocular, etc. administration. Two advantages of drug–dendrimer conjugates over drug/dendrimer complexes and traditional drug dosing are prolonged lifetime and more stable level of the active substance (Fig. 18) (Cheng et al. 2008b; Cheng and Xu 2008). Complexation of sulfadiazine with PAMAM dendrimer led to increase of anti-toxoplasmic activity up to 7 orders of magnitude (Cheng and Xu 2008; Prieto et al. 2006).

The crucial role of the guanidinium functional group in facilitating the transport of dendritic polymers through liposomal and cell membranes has been reviewed (Theodossiou et al. 2008).

Controlled drug release behavior of hyperbranched polyamidoamines (HPMA) in the presence of β-CD was

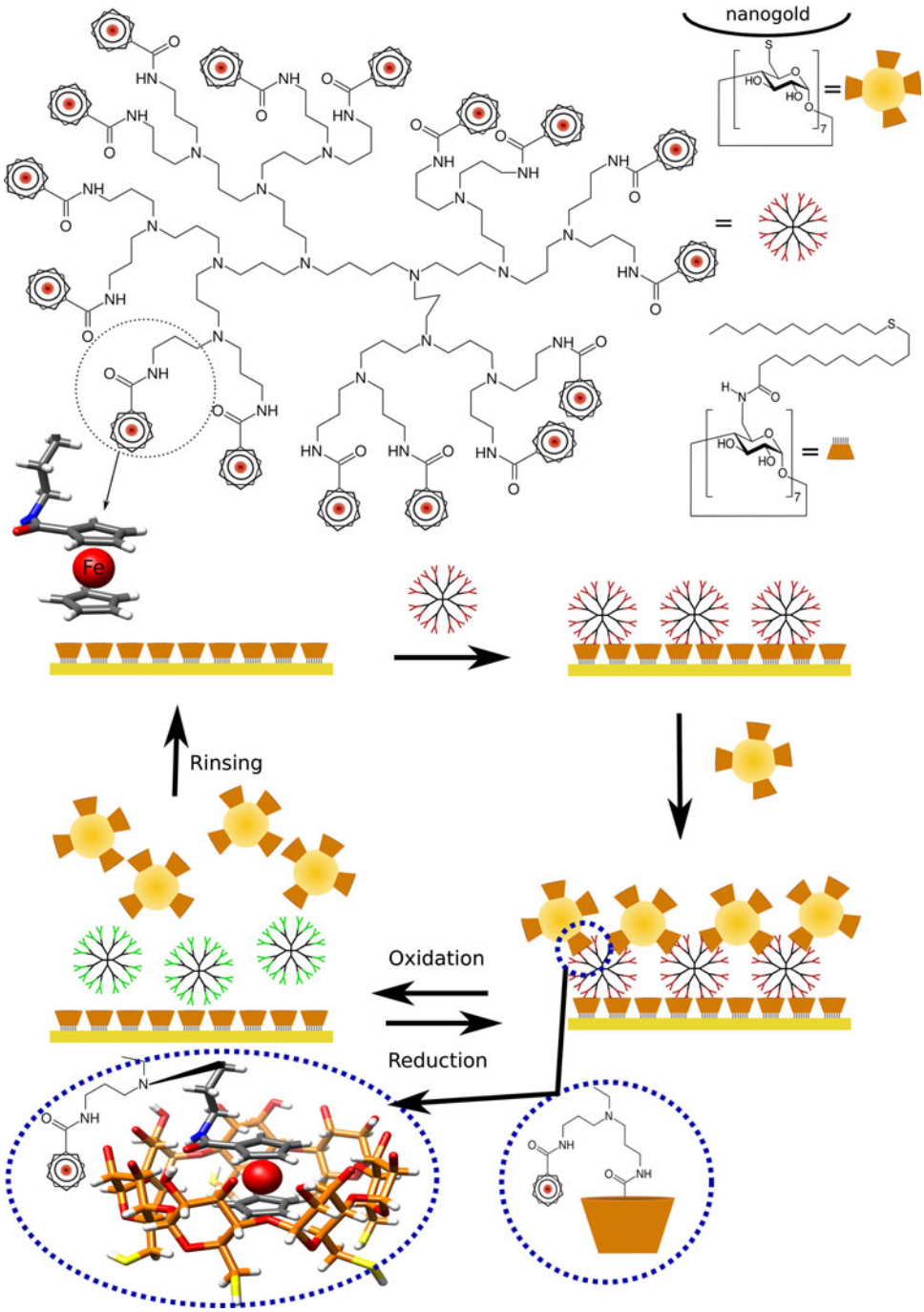

Fig. 17 Schematic representation of an important goal of nanotechnology, i.e. regenerable surfaces and reversible attachment of nanostructures (Ling et al. 2008)

studied. Three HPMA-β-CDs and a pure HPMA were synthesized (Zhou et al. 2009) by Michael addition polymerization. The drug release was tested on an anticancer drug chlorambucil as model, which was loaded into them via a solution method for in vitro release studies. The loading of chlorambucil into these dendrimers leads to an evident increase in their glass transition temperatures, and ΔT_g depends on the β-CD content. The controlled release experiments have proven that the presence of β-CD in the dendrimer can appropriately slow the release of chlorambucil from HPMA-β-CDs.

Agrawal et al. (2007) synthesized [Lys_8-Lys_4-Lys_2-$Lys]_2$-PEG MAPs (G4) having PEG-1000 as a core. Galactose was selected as model sugar for coating of these MAPs. Transmission electron microscopy (TEM), IR, NMR, and MALDI TOF mass spectroscopic studies were used for characterization of both uncoated and galactose-coated MAPs. Chloroquine phosphate (CP)-loaded

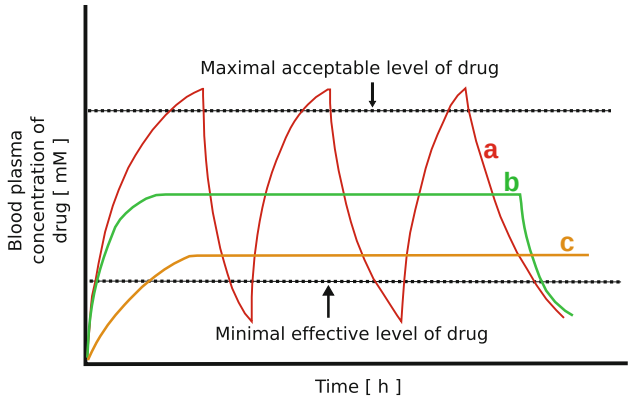

Fig. 18 Pharmacokinetics during therapy (Cheng et al. 2008b; Cheng and Xu 2008). **a** Traditional drugs. **b** Drug–dendrimer complexes. **c** Drug–dendrimer conjugates

uncoated and coated MAPs were studied for in vitro hemolytic toxicity, drug release rate, and stability. Ex vivo cellular uptake studies of uncoated and coated drug MAPs in macrophages revealed almost 5 times reduced phagocytosis due to galactose coating ($p < 0.0001$). In vitro–in vivo release behavior indicated ability of galactose-coated drug dendrimers formulation in controlled drug delivery of CP. The hemolytic toxicity of galactose-coated formulations was drastically reduced compared to uncoated MAP as well as plain drug. Galactose-coated formulations are less immunogenic compared to uncoated formulations. Generally, galactose-coated MAPs can be applied for controlled delivery of CP much more safely in comparison with its uncoated formulation both in vitro and in vivo.

Electron paramagnetic resonance (EPR) spectroscopy was used for studies of dendrimeric carriers for drug delivery (Martini and Ciani 2009). EPR can be helpful in the analysis of the guest–host behavior by using classical nitroxides as probes for screening of drug capture and release. Dependence on the dendrimer generation and on their interactions with molecular species was investigated.

Since a wide variety of tumors overexpress fibroblast growth factor receptor (FGFR), it is an important target for drug delivery (Thomas et al. 2010). The specific binding and internalization of the conjugate of FGF-1 and G5-PAMAM labeled with FITC were proven by flow cytometry and confocal microscopic analysis. The excess of non-conjugated FGF-1 completely suppressed the binding and uptake of FGF-conjugated dendrimers. Nuclear and cytosolic localizations were observed; therefore these conjugates can serve as a platform for drug delivery in specific tumor cell compartments, and as an FGF delivery agent for angiogenesis and wound healing.

An influence of dendrimer generation and PEG length on the biodistribution of PEGylated dendrimers was studied (Kojima et al. 2010). Diethylenetriaminepentaacetic acid (chelating agent) was conjugated to PEGylated PAMAM and labeled with radioactive indium. This conjugation led to prolonged blood circulation and avoided the accumulation in normal organs including the kidneys and the liver.

In vitro evaluation and in vivo tumor accumulation of PEGylated PAMAM dendrimer–doxorubicin conjugates were studied (Zhu et al. 2010). The highest PEGylation degree led to a potential candidate for solid tumor treatment.

The ring-opening polymerization and the direct atom transfer radical polymerization were used for synthesis of star PAMAM-b-poly(ε-caprolactone)-b-poly(gluconamidoethyl methacrylate) block copolymers (Daia et al. 2009). Nanoparticles formed from these copolymers were studied as carriers of nimodipine and as biomolecular binders of ConA with the aim to develop a drug against hemorrhage.

The G2 and G3 of dendrimers containing naproxen in the core and Asp oligopeptides in the periphery were synthesized (Ouyang et al. 2009). These conjugates possessed a high affinity to hydroxyapatite in vitro and pave the way for the use of peptide dendrimers for bone targeting.

Mesoscopic simulations (Krafczyk et al. 2005) were used for description of charged dendrimers permeability across lipid bilayer membrane (Yan and Yu 2009). The structures of membranes are modulated by adjusting their surface tensions. The simulations have proven that the permeability of charged dendrimers were enhanced in the tense membranes, and the permeability in the actual hole was significantly increased in comparison with that in the lipid poor section. The mechanism of charged dendrimer-induced pore nucleation in the tense membranes is discussed. Partitioning of PAMAM dendrimers between *n*-octanol and water were studied (Giri et al. 2009).

The topics of dendrimer solubility (both dendrimers as such and their influence to drug solubility), drug delivery, dendrimer biocompatibility, and toxicity are closely related and influence each other.

Dendrimers in gene delivery

Nucleic acids are delivered to particular target sites using a number of viral and non-viral delivery systems, both of which have distinct advantages and disadvantages (Dutta et al. 2010; Dufes et al. 2005; Paleos et al. 2007; Yellepeddi et al. 2009). In comparison with viral vectors, the non-viral ones offer the advantage of safety and flexibility. On the other hand they have lower efficiency. Principal properties, such as defined architecture and a high ratio of multivalent surface moieties to molecular volume make these dendritic nanoscale materials very important

for the development of synthetic (non-viral) vectors for nucleic acid delivery (Dufes et al. 2005).

Dendrimers can interact with various forms of nucleic acids, such as plasmid DNA, antisense oligonucleotides, and RNA. These interactions lead to complexes, which protect the nucleic acid from degradation. The nature of interactions between the dendrimers and the nucleic acids is mainly electrostatic where the cationic dendrimer binds the anionic nucleic acids. The transfection efficiency is determined with the net positive charge of the dendrimer nucleic acid complex. On the other side, highly cationic complexes are very cytotoxic. The properties of these complexes depend on many factors, such as stoichiometry, concentration of dendrimer amines and nucleic acid phosphates, bulk solvent properties like pH, salt concentration, buffer strength, etc. (Dutta et al. 2010; Dufes et al. 2005).

The use of PAMAM dendrimers and their modifications in gene delivery were reviewed (Paleos et al. 2007, 2009; Yellepeddi et al. 2009). Micellar glycocluster nanoparticles were used as artificial viruses for gene delivery. For details of size-controlled gene coating with glycocluster nanoparticles, see Mastrobattista et al. (2006), Aoyama et al. (2003), Aoyama (2004, 2005), Boas et al. (2006f), Niederhafner et al. (2008a).

PAMAM dendrimers with pentaerythritol core (PEC) with 12 branches were synthesized (Wang et al. 2009). These PEC dendrimers (G3–G5) efficiently condensed DNA into nanoscale complexes with slightly positive charges. The transfection efficiency in different cell lines was determined. The PEC dendrimers possessed higher transfection efficiency and much lower cytotoxicity, in comparison with the commercial non-viral gene carriers PEI, MAP, and PAMAM dendrimers with an ethylenediamine core (G5–G7). Thus, the PEC dendrimers can serve as novel non-viral gene carriers.

Lyulin et al. (2008) used molecular dynamics approach to shed light on how the strength of electrostatic interactions affects the properties of dendrimer chain complexes often used as a means in drug and gene delivery. They used general polyanion as a simple model of nucleic acid and a cationic PAMAM dendrimer.

Molecular dynamics (MD) proved that a linear polyelectrolyte forms a complex with the dendrimer showing a remarkable condensation of the supramolecule. During this complexation, considerable dehydration of the chain was observed. The dehydration is more pronounced, when the electrostatic interactions strengthen. The charged dendrimers have the ability to efficiently compress and protectively screen guest chains from the surrounding medium. These two well-known prerequisites enable vehicle-mediated delivery of drugs and genes into cells.

PAMAM-G3 was conjugated with α-CD (Tsutsumi et al. 2008). These conjugates served as carrier of short hairpin RNA (shRNA) expressing plasmid DNA (shpDNA). The conjugates suppressed the enzymatic degradation of shpDNA by DNase I.

The use of dendrimers with β-CD cores for gene delivery was described (Diaz-Moscoso et al. 2009; Xu et al. 2009) (for more details see "Cyclodextrins").

A novel host family based on bis-(guanidinium)-tetrakis-(β-CD) tetrapod was described (Menuel et al. 2008). This family of compounds served as efficient non-viral vector for transfection of siRNA and DNA to human embryonic lung fibroblasts.

Structural analogs of PAMAM-hyperbranched poly (amido amine) dendrimers (HPAMAM) were studied as an effective agent for gene delivery (Wang et al. 2010b). The HPAMAM was modified on the terminal amino groups with phenylalanine to various degrees (HPAMAM-PHE_{30}, PHE_{45}, PHE_{60}). The HPAMAM and HPAMAM-PHEs formed complexes with plasmid DNA (pDNA) at various mass ratios. The HPAMAM-PHE_{60} was the most efficient transfection agent in comparison with commercial transfection reagent PEI and other compounds from this series. The HPAMAM-PHE_{60} can be good material for gene delivery and other applications because of its large-scale availability, economical cost, and low toxicity.

Transfection experiments of polycationic phosphorous dendrimers, which form complexes with DNA, were carried out (Padie et al. 2009). These dendrimers were effective transfection agents with low toxicity.

The above-mentioned examples clearly demonstrate the practical utility and potential of dendrimers in the field of gene delivery.

Carbohydrate interactions of glycopeptide dendrimers

Nanodimensional macromolecular assembly of complex, highly branched glycoconjugates, which surround every cell is referred to as a cell's glycocalyx (Shaikh et al. 2008; Kleinert et al. 2004, 2008; Patel and Lindhorst 2006; Heidecke and Lindhorst 2007). This layer plays a key role in molecular recognition and interactions of protein receptors, such as lectins, selectins, and their carbohydrate ligands. Interactions with the glycocalyx are of basic importance for many biological processes, such as cell–cell recognition, immunological response and fertilization, as well as metastasis, microbial adhesion, inflammation, and other disease states of a cell or tissue. Glycodendrimers are a perfect tool to study biological processes occurring on cell surfaces, such as bacterial adhesion. Glyco-self-assembled monolayers (glyco-SAMs) can play a role of glycocalyx model. Those glyco-SAMs are suitable for binding assays using SPR (Kleinert et al. 2008).

Bacteria

Bacteria become resistant toward common antimicrobial agents. This worldwide problem is calling for immediate solution. Diseases caused by resistant organisms lead to adverse clinical outcomes, increased mortality, and have a deep economical impact. Some of the infectious diseases are started by the binding of pathogenic bacterial lectins to host cells glycoconjugates. The most common type of adhesive anchors in *E. coli* and several other enterobacteria are Type 1 fimbriae, i.e. 30 kDa lectin-like subunit FimH, which mediate mannose-specific binding (Roy and Touaibia 2007; Sperling et al. 2007; Patel and Lindhorst 2006; Pieters 2007). Mannopyranoside residues including high-mannose oligosaccharides can be bound by many proteins, such as plant lectins, particularly ConA, *Dioclea grandiflora*, and pea lectins (Chabre and Roy 2010; Roy and Touaibia 2007; Chabre and Roy 2008; Imberty et al. 2008; Touaibia and Roy 2007; Roy 2003). Multivalency of the receptors is caused by oligomerization of lectin domains and/or multipresentation on pathogen surface. The efforts for understanding molecular basis of strategies used by microbes for adhering to host glycans, and the related processes in producing glycodendrimers and glycomimetics that could block the adhesion was reviewed (Roy and Touaibia 2007; Chabre and Roy 2008; Imberty et al. 2008; Touaibia and Roy 2007; Pieters 2007). In order to find a way to new antibiotics it is necessary to understand the adhesion phenomena on a molecular basis. Glycomimetics could serve as inhibitors of these recognition events and prevent adhesion and colonization of host tissues by pathogens (Fig. 19) (Roy and Touaibia 2007; Chabre and Roy 2008; Imberty et al. 2008; Touaibia and Roy 2007; Sperling et al. 2007; Patel and Lindhorst 2006). These interactions measured on a per saccharide basis are too weak (mM). To overcome this hurdle glycodendrimers can address that issue. For more details see "Cluster effect".

The topic of α-mannose glycodendrimers and detailed descriptions of design and applications of mannosylated inhibitors against fimbriated type 1 *E. coli* was reviewed (Roy and Touaibia 2007; Touaibia and Roy 2007; Chabre and Roy 2008; Imberty et al. 2008; Patel and Lindhorst 2006). Design of dendrimeric glycopeptide vaccines against several bacteria has been reviewed (Rojo 2009).

Oligomannoside mimetics were synthesized by mannosylation of spacered glucose and oligosaccharide (α,α-trehalose, β-melibiose, raffinose) cores (Dubber et al. 2006b). These carbohydrate centered (octopus) mannosides were tested by ELISA as inhibitors of mannose-specific bacterial adhesion mediated by type 1 fimbriae (*E. coli*). Their relative inhibitory potencies (RIP, for methyl α-mannoside = 1) and IC_{50} values were compared with a series of mannobiosides and finally with the polysaccharide mannan. The RIP values suggest a new interpretation of the mechanism of bacterial adhesion according to a macromolecular rather than multivalency effect.

The same authors (Sperling et al. 2007) prepared clustered glycomimetics as model compounds to study multivalent interactions with glycocalyx constituents. The synthesis of dodecavalent octopus neoglycoconjugate using squaric acid diester mediated coupling and tris(2-aminoethyl)amine as a core afforded dendrimeric dodecavalent cluster mannoside with 12 α-mannose units. This compound was tested for anti-adhesive properties in an ELISA which allows detection of the adhesion of type 1 fimbriated *E. coli* to a mannan-coated polystyrene surface. The relative inhibitory potency (RIP) of this dendrimer was 190 in comparison with methyl α-mannoside (RIP = 1).

Nonavalent cluster mannosides were synthesized starting from a number of trivalent, branched molecular wedges. The chemical characteristics of their spacer moieties and spacer lengths were varied (Patel and Lindhorst 2006). The trivalent dendrons were connected to the target nonavalent structures by a peptide bond by means of HATU

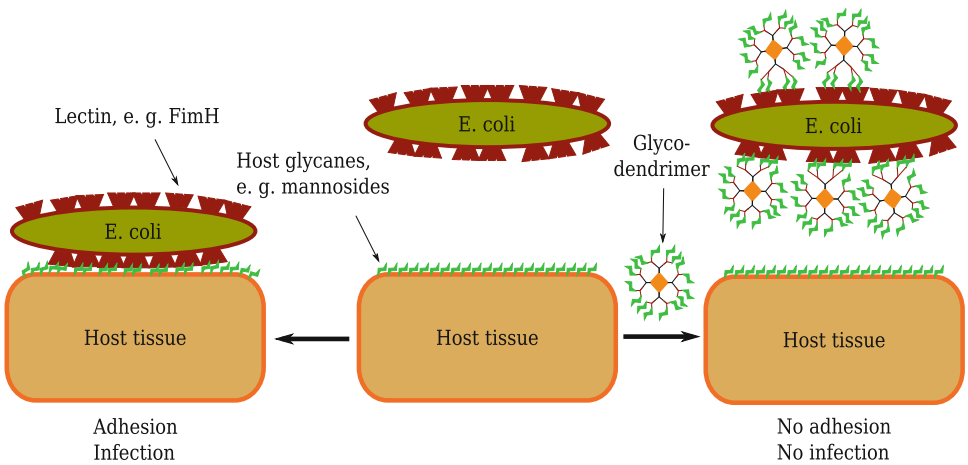

Fig. 19 Role of glycodendrimers in prevention of host colonization by bacteria (Touaibia and Roy 2007; Pieters 2007)

reagent. The prepared nonavalent glycopeptide dendrimers containing methyl α-mannopyranose were tested as inhibitors of the *E. coli* type 1 fimbriae-mediated adhesion using ELISA. Regrettably, very poor or no inhibitory activities were found.

To cast light up to glycocalyx interactions, the same group (Kleinert et al. 2004) used trivalent MAGs containing L-fucose and mannose, respectively, coupled to thio-functionalized alkane and alkane-oligoethylene glycol spacers with the aim to study the formation of SAMs on gold. These MAGs can be assembled on gold wafers to serve as glycocalyx mimetics. See also Shaikh et al. (2008).

To develop new mannosylated dendrimers as potential drug candidates for gastrointestinal and urinary tract infections caused by *E. coli*, Roy et al. (Touaibia and Roy 2008) developed a short and efficient strategy for the first synthesis of "Majoral-type" multivalent glycodendrimers bearing covalently bound α-mannopyranosides onto a cyclotriphosphazene scaffold assembled using single-step Sonogashira and 1,3-cycloaddition click chemistry (Fig. 20). The authors present the syntheses of representative members of a new class of glycodendrimers that are

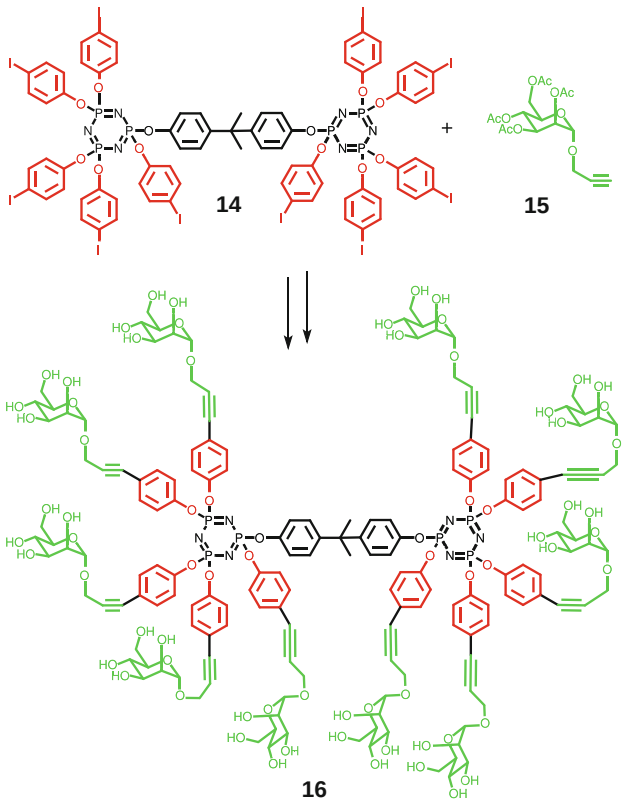

Fig. 20 Synthesis of "Majoral-type" multivalent glycodendrimers bearing covalently bound α-mannopyranosides onto a cyclotriphosphazene scaffold assembled using single-step Sonogashira coupling (Touaibia and Roy 2008)

built on non-toxic cyclotriphosphazene cores. Cyclotriphosphazene-centered dendrimers are the landmark of the Majoral's group (Servin et al. 2007). The whole set of α-mannopyranoside dendrimers with valencies ranging from 6 to 18 units and different epitope spatial arrangements was tested using the well-established ConA. A kinetic turbidimetric assay was used to study the relative ability of these mannosylated dendrimers to act as cross-linking reagents. The polysaccharide yeast mannan was used as the positive control. The time course of ConA precipitation by the corresponding mannosylated dendrimers was studied. Using ConA, mannosylated dendrimers with the same hexameric valency, differing by the mannoside spacer arm (aryl or triazole), showed highly equivalent protein-binding properties compared to those of the positive control yeast mannan. The decavalent dendrimer (**16**) (Fig. 20) containing alkyne spacer was the fastest and more complete in forming the insoluble cross-linked lattice. Surprisingly, dodecamer and octadecamer, having a slightly longer spacer arm and more flexibility than (**16**), showed lower cross-linking potencies. The authors (Touaibia and Roy 2008) explain the noticeable crosslinking enhancement observed for (**16**) by the "existence of more favorable extended intersugar distances, thus facilitating entry into the carbohydrate's active site and permitting a higher protein cross-linking ability". These new α-mannopyranoside dendrimers are promising tools as probes or effectors of biological processes involving multivalent carbohydrate-binding proteins.

Iterative synthesis of spacered glycodendrons as oligomannoside mimetics was elaborated (Heidecke and Lindhorst 2007) with a new approach in glycodendrimer synthesis, in which a 3,6-diallylated carbohydrate is utilized as core molecule, hydroboration–oxidation is the activating step, and glycosylation with branched and unbranched sugar trichloroacetimidates is used for dendritic growth. The six new hyperbranched glycodendrons were tested for their potential as inhibitors of *E. coli* type 1 fimbriae-mediated bacterial adhesion in an ELISA. The results were interpreted with regard to sugar valency and spacer characteristics.

Di-, tetra-, and octavalent dendrimers containing galactose were prepared via click chemistry. They inhibit cholera toxin binding as strongly as the natural ganglioside GM1 oligosaccharide does (Branderhorst et al. 2007). This result is important for cholera toxin therapy and detection.

Multivalent GM1os and GM2os dendrimers were prepared (Pukin et al. 2007) by using efficient click chemistry with dendritic scaffolds that had extended arms. The unambiguously characterized dendrimers showed strong multivalent binding to cholera toxin B-subunit (CTB5), with an excellent value of at least 380,000-fold stronger binding for octavalent GM1os dendrimer than monovalent

GM1os derivatives. This can be exploited for development of very sensitive sensor applications.

A series of glycosylated PePOs bearing 4, 6, 8, and 10 L-fucose residues were prepared (Morvan et al. 2007) and the binding to the L-fucose-specific bacterial lectin (PA-IIL) was determined through an enzyme-linked lectin amplification competition assay. The IC_{50} values measured are 10–20 times better than for monovalent L-fucose. The enhancement observed cannot be attributed to a cluster effect, because the relative activity per carbohydrate is only of 2 for every glycocluster, regardless of the number of L-fucose residues. The increased binding of these glycoclusters is most probably caused by the recently described "macromolecular" effect (Dubber et al. 2006b).

Viruses

Influenza virus is a highly pathogenic virus (Sakamoto et al. 2009; Oka et al. 2009). About 230,000 patients with influenza virus are hospitalized every year in the USA. The worst influenza pandemy was Spanish flu in 1918–1919, which caused death of at least 20 million people. In a case of a new type of pandemic flu, it is estimated that about 62 million people will die worldwide. Influenza A viruses contain two unique glycoproteins, hemagglutinin (HA) and neuraminidase (NA), on their surfaces. HA and NA play key role in infection and replication. To prevent the first contact of the virus and a host cell, the first approach has focused on HAs, which participate in infection of influenza virus to host cells. Sialyl lactose (Neu5Ac-α-(2 → 3)-Gal-β-(1 → 4)-Glc) is known as a specific receptor of HAs of influenza A viruses. On the basis of carbohydrate–protein interaction, HA plays an important role in adhesion of influenza viruses to the surface glycosyl receptor of the host cell virus (Sakamoto et al. 2009; Oka et al. 2009). Dendrimers containing sialic acid as synthetic inhibitors of influenza virus have been recently reviewed (Carlescu et al. 2009).

To develop novel influenza sialidase inhibitors, Japanese authors (Sakamoto et al. 2009) prepared carbosilane dendrimers composed of 12 types of sialylated dendrimers with thioglycosidic linkage that are resistant to hydrolysis by the sialidases. Dendrimer scaffolds with 3-, 4-, 6-, and 12-functionalized dendrimers, and different spacer patterns, i.e. aliphatic linkage, ether and amide linkages were synthesized. Biological evaluations of these glycodendrimers showed that all of the ether and amide elongated compounds had inhibitory potencies for the influenza sialidases in the mM range.

The same group (Oka et al. 2009) elaborated a synthesis of a series of carbosilane dendrimers uniformly functionalized with Neu5Ac-α-(2 → 3)-Gal-β-(1 → 4)-Glc moieties. These compounds were systematically tested for anti-influenza virus activity. The tested glycodendrimers had unique biological activities depending on the form of their core frame, and Dumbbell(1)6-amide type glycodendrimer showed particularly strong inhibitory activities against human influenza viruses [A/PR/8/34 (H1N1) and A/Aichi/2/68 (H3N2)]. The results suggested that dumbbell-shaped dendrimers were found to be the most suitable core scaffolds in this study. It led to improved inhibition activities in the μM level. These compounds are promising therapeutic agents for influenza disease.

The HIV envelope glycoprotein gp120 uses the high-mannose clusters on its surface for targeting of the C-type lectin on dendritic cells (DC-SIGN). In order to design carbohydrate-based antiviral agents, mimics of the cluster presentation of oligomannosides on the virus surface were developed (Martinez-Avila et al. 2009). A library containing multivalent water-soluble gold glyconanoparticles (manno-GNPs) presenting truncated $Man_9GlcNAc_2$ was synthesized and tested as inhibitors of DC-SIGN binding to gp120. These manno-GNPs are ligands for DC-SIGN, which also interfere with the early steps of other infections through specific recognition of associated glycans. (Oligo)mannosides with different thiol containing spacers serving for attachment of the glycoconjugates to the gold surface were prepared. Their inhibition potency toward DC-SIGN binding to gp120 was studied by SPR. The tested manno-GNPs completely inhibit the binding in the micro- to the nanomolar range. The corresponding monovalent mannosides required millimolar concentrations. The best inhibitors of gp120 binding to DC-SIGN are manno-GNPs containing the disaccharide α-Man*p*-(1 → 2)-α-Man*p*. They showed roughly 20,000-fold increase of the activity in comparison with the corresponding monomeric disaccharide.

A series of catanionic multivalent analogs of GalCer were described (Perez-Anes et al. 2010). Dendrimers based on cyclotriphosphazene core with phosphonic acid in the branches formed non-covalent complexes with *N*-hexadecylamino lactitol moieties. These supramolecular systems showed anti-HIV1 activity. They have submicromolar IC_{50} in a cell-based HIV-infection model, but also a high general cytotoxicity.

Dendrimeric glycopeptide vaccines against several viruses were reviewed (Rojo 2009; Gajbhiye et al. 2009; Niederhafner et al. 2008c).

Cancer

Dendritic cells (DC) play a key role in development of therapeutic cancer vaccines, because they have a unique ability to display tumor epitopes via the MHC class I pathway inducing cytotoxic $CD8^+$ T lymphocyte responses. DC-SIGN and the mannose receptor are membrane lectins on the surface of DCs which recognize oligosaccharides-containing mannose and/or L-fucose and

mediate sugar-specific endocytosis of synthetic oligolysine-based glycoclusters.

Roche et al. (Srinivas et al. 2007) designed and synthesized glycocluster conjugates containing a $CD8^+$ epitope of the Melan-A/Mart-1 melanoma antigen. The glycocluster–Melan-A conjugates were prepared by coupling glycosynthons: oligosaccharyl-pyroglutamyl-β-alanine derivatives containing either disaccharides, a dimannoside (Man-α-(1 → 6)-Man) or lactoside, or Lewis oligosaccharides Le^x and Le^a, to Melan-A 16-40 peptide Ac-G^{16}HGHSYT-TAE$E^{26}LAGIGILTV^{35}$-ILGVL^{40}KKKK, (Ac, for acetyl) containing a variant of the HLA-A2-restricted $CD8^+$ epitope (26–35) *ELAGIGILTV* instead of EAAGIGILTV, ended by a tetralysine tail. Confocal microscopy and flow cytometry have shown that fluorescent-labeled Melan-A glycoclusters containing either dimannoside or Lewis oligosaccharide were taken up by DC and concentrated in acidic vesicles, contrary to lactoside glycopeptides, which were not at all taken up. SPR showed that dimannoside and Lewis–Melan-A conjugates bind MR and DC-SIGN tightly. DCs with these conjugates elicit a $CD8^+$ T-lymphocyte response and they can serve as a promising tool for the development of tumor vaccines.

The use of dendrimeric glycopeptides against cancer including anticancer vaccines was reviewed (Tekade et al. 2009b; Zhu et al. 2009; Niederhafner et al. 2008c; Wolinsky and Grinstaff 2008).

Other examples

Carbohydrate–carbohydrate interactions (CCIs) between cell surface glycans mediate cellular recognition and adhesion. Multivalent glycoconjugates were prepared (Seah et al. 2009) based on G4 PAMAM dendrimers. Their interaction with Langmuir monolayers containing GM3 was investigated. Excessive carbohydrate valency adversely affects the CCI. The interaction of GM3 monolayer with lactose-functionalized dendrimers is selective in the presence of calcium ions. These results provide the first example of the use of glycodendrimers as model systems for studying CCI. Therefore, these glycodendrimers may serve as useful agents for probing CCI in vivo and can also find application as targeted diagnostic and antimetastatic agents.

To study differential effects of oligosaccharides on the hydration of simple cations (Eriksson et al. 2008) an octasaccharidic branched *N*-glycan of the high-mannose type $Man_6GlcNAc_2$ was chosen. Molecular dynamics simulations were performed (Eriksson et al. 2008) to explain and understand solvation properties of simple cations of biological relevance (Na^+, K^+, Mg^{2+}, and Ca^{2+}) in explicit water, near single and multiple oligosaccharides as glycocalyx models. The studied oligosaccharide prefers direct contact with K^+ over Na^+. On the other side the Na^+ contacts are longer lived. The observed interactions lead also to strong, but short-lived changes in oligosaccharide conformations, with oligosaccharides packed around K^+ with multiple contacts. These results are important for better understanding of glycocalyx functions and interactions.

The molecular level analysis of cell surface phenomena was studied by controlled addition of structurally defined components to live cell membranes (Rabuka et al. 2008). Cell surfaces were engineered to display synthetic bioactive polymers at defined densities by exogenous membrane insertion. The polymers were designed to mimic native cell-surface mucin glycoproteins (this structure can be termed brush glycodendrimer, see Niederhafner et al. 2008b). Incorporation into the membranes of live cultured cells was enabled by end-functionalization with a hydrophobic anchor. The dynamic behavior of cell-bound glycopolymers bearing various hydrophobic anchors and glycan structures was studied by fluorescence correlation spectroscopy (FCS). Interactions of proteins with these artificial receptors were studied. These artificial receptors behaved similarly to mucins, i.e. they showed specific protein binding and internalization through endocytic pathways. Both polymer density and mobility can be visualized by FCS, which provides a powerful tool of molecular characterization in a synthetically engineered cell-based system. Polymer insertion efficiency and mobility were found to be independent of glycan structure. This method allows separation of effects caused by changes of glycan structure, density and dynamics. This system enables to study cell-surface phenomena with such a degree of chemical control which cannot be achieved by common biological tools.

Dendrimers with up to eight α-mannose moieties were prepared by click chemistry (Branderhorst et al. 2008). The attachment to aluminum oxide chips was done via a spacer that was linked to the dendrimer core. Binding of the fluorescent lectins ConA and GNA to the glycodendrimer chips was observable in real time. It was possible in a single experiment to observe the multivalency enhancement or cluster effect in the binding event. For ConA, this effect was small in agreement with its widely spaced binding sites, whereas it was large for GNA, with its twelve much more closely spaced binding sites. Detailed discussion about the dependence of cluster effect on differences between the structure of ConA and GNA is given. These dendrimer-fitted chips represent a valuable tool for screening multivalency effects, including kinetic and thermodynamic data on binding events. Inhibition experiments are also possible.

Jin et al. (2006) prepared glycopeptide dendrimers based on peptoids containing β-Ala. They contained four copies of a trisaccharide and one dansyl group as fluorescence

label. The trisaccharide is derived from a major antigenic epitope in pectic polysaccharides from *Bupleurum falcatum* L. The roots of this plant (Japanese name *Saiko*) are used in Chinese and Japanese herbal medicine for the treatment of chronic hepatitis, autoimmune diseases, and nephrotic syndrome.

Self-assembled peptide microtubes were covalently (by means of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, and *N*-hydroxysuccinimide) attached to chitosan (Henricus et al. 2009). The morphology of the chitosan assembled on the surface of the microtubes can be tuned by altering the pH of the growth solution. Cytotoxicity studies carried out in the presence of mouse embryonic fibroblasts indicate that the chitosan microtubes are highly biocompatible. The cells are able to survive and proliferate at a similar rate to the control. Antibacterial activity against *E. coli* proved that the chitosan bound microtubes are bactericidal. Possible application in development of highly efficient antibacterial materials is stressed. Chitosan based dendrimers were reviewed (Mourya and Inamdar 2008).

Dendrimers as anti-prion agents

Prions are suspected as causative agents of several neurodegenerative diseases, such as scrapie, bovine spongiform encephalopathy, Creutzfeld–Jakob's disease, Gerstmann–Sträussler–Scheinker's syndrome, etc. PrP^{Sc} is a protease-resistant, aggregated, misfolded conformer of the cellular prion protein (PrP^{C}). PrP^{Sc} aggregates are accumulated in neuronal tissue, especially in the brain as a constant feature of all prion diseases. According to the "prion hypothesis", PrP^{Sc} is the sole agent responsible for transmission and pathogenesis of the prion diseases (Prusiner 1998; Aguzzi 2008; Gilch et al. 2008). Natural and synthetic prion structures were studied by X-ray fiber diffraction (Wille et al. 2009). PrP^{Sc} is remarkably stable toward a range of chemical and physical treatments (Taylor 2003); however, its infectivity is completely lost when it is exposed to high concentrations of protein denaturants like guanidinium and thiocyanate ions (Prusiner et al. 1981). Inactivation of PrP^{Sc} can be also achieved by acidic SDS treatment (Peretz et al. 2006). Since PrP^{Sc} inactivation and dissociation proceed *via* a solubilization of PrP^{Sc}, its solubilizers can serve as a suitable model for PrP^{Sc} inactivation. Peptide β-sheet breakers (Soto et al. 2000) reduced PrP^{Sc} infectivity by interfering with the transition of PrP^{C} to PrP^{Sc} and by (partially) unfolding of PrP^{Sc}. The dendrimers described by Supattapone and coworkers (Supattapone et al. 1999a, 1999b, 2001) are very potent inactivators of preexisting PrP^{Sc}. The G5-PAMAM dendrimer (carrying 64 surface primary amino groups) at 0.1 μM completely prevented PrP^{Sc} caused toxicity on ScN2a cells. The presence of dendrimers makes fibrils vulnerable to protease K degradation. The mechanism of destabilization of PrP^{Sc} aggregates is not known. Formation of polycations on the dendrimer surface at a low pH increases the efficiency of the PrP^{Sc} dissolution (Cordes et al. 2007; Supattapone et al. 2009). The proteins interact with both dendrimer interior and surface (Giehm et al. 2008). According to pH-dependence, the interactions are mainly caused by electrostatics as confirmed by studies on four other proteins. The precipitation and destabilization are positively correlated indicating that surface immobilization of denaturing groups profoundly affects its interactions with proteins. These dendrimers can be potentially used as reagents for prion decontamination, i.e. removal of prions from skin, surgical instruments, surfaces, etc. (Rojo and Delgado 2007).

Both cationic dendrimers and PrP^{Sc} molecules accumulate in lysosomes, where the acidic environment facilitates dendrimer-mediated PrP^{Sc} disaggregation (Supattapone et al. 2009). A range of different amyloid proteins can be disaggregated by dendrimers. Dendrimers can break preformed fibers and cap elongating fibers on model peptides. An application of dendrimers as therapeutic compounds for neurodegenerative disorders of protein misfolding include limited spectrum of activity, poor bioavailability, and detrimental neurological side effects.

G1–G5 PPI dendrimers were synthesized with guanidine- (positively charged at neutral pH) and urea-functionalized (uncharged) surfaces (Cordes et al. 2007). The PrP^{Sc}-solubilizing effect of these dendrimers was studied in a SMB cellular system infected with the prion disease. These dendrimers and the unmodified ones had comparable effect in PrP^{Sc} clearance. The activity is generation dependent and the higher generation of dendrimer the higher activity. Urea functionalization led to partially less active dendrimers, which are much less cytotoxic. Guanidine- and non-functionalized dendrimers are more effective, however, extremely cytotoxic. The most efficient PPI dendrimers (G4 and G5) were unmodified and guanidino modified, which cleared PrP^{Sc} completely by incubation for 4 days at less than 50 nM. Therapeutic uses of modified dendrimers can be suitable due to the low effective concentrations.

Heparin plays an important role in the pathogenesis of prion diseases, affecting the process of fibril formation. Interactions between dendrimers and heparin and their implications for the anti-prion activity of dendrimers were studied (Klajnert et al. 2009). An acceleration or inhibition of prion fibrillogenesis depends on heparin concentration: human prion protein 185–208 (HuPrP(185–208)) aggregates in the presence of optimal amount of heparin, but concentrations ten times lower or higher cause no aggregation. PAMAM, PPI, and phosphorous dendrimers with

anti-prion activity interact with heparin. Cationic dendrimers and anionic heparin interact mainly electrostatically. These interactions are indirectly responsible for the inhibition or enhancement of fibril formation by dendrimers.

Maltose-modified PPI dendrimers were prepared by reductive amination of unmodified G2–G5 PPI dendrimers in the presence of excess maltose (Klajnert et al. 2008). These dendrimers possess enhanced molecular rigidity and the maltose units on the surface have hydrogen-bond-forming properties. Encapsulation of 1-anilinonaphthalene-8-sulfonic acid and biological properties, such as hemolysis and interactions with human serum albumin (HSA) and HuPrP(185-208) were carried out with the modified and unmodified dendrimers. The maltose modified dendrimers bury a low molecular weight fluorescent dye by means of a dendritic box effect, in contrast to the interfacial uptake characteristic of the unmodified PPI dendrimers. Maltose modified dendrimers are significantly less toxic. The parent compounds and modified dendrimers interacted with HSA with comparable activity. They have potential to be applied as anti-prion agents. The desirable properties of non-toxic anti-amyloid agents with no cationic charges can be obtained to initiate the next development of more efficient anti-amyloid agents.

The interactions of the peptide Aβ(1–28) involved in Alzheimer disease and the HuPrP(106–126) peptide suspected to be preferentially involved in spongiform encephalopathies with three different types of dendrimers were studied by spin-probe and spin-label techniques (Klajnert et al. 2007a). The interactions between dendrimer and peptide monomer are stronger for Aβ(1–28) than those for HuPrP(106–126). PAMAM dendrimers suppress the aggregation of the peptides more than PPI dendrimers do.

Since there is a positive correlation of anti-prion activity with charge density, quaternization of amine groups of G4 PEI and PAMAM dendrimers was carried out in order to reduce the intrinsic cytotoxicity of polyamines and to increase the charge density in the compounds (Lim et al. 2010). The quaternization led to improvement of the cytotoxicity profile of the dendrimers; however, it slightly reduced their anti-prion activity. Besides, quaternized dendrimers inhibited prion propagation facilitated by conversion of PrP^{C} to PrP^{Sc} without dramatic loss of activity. The structural control of dendrimers can provide another space for improvement, because cytotoxicity profile and anti-prion activity strongly dependent on the structure of dendrimers. The quaternization represents a useful strategy for developing non-toxic dendrimers with potent anti-prion activity.

Cationic phosphorous dendrimers were studied as the inhibitors of the prion peptide PrP(185–208) aggregation using a spectrofluorometric assay with thioflavin T (ThT) and Fourier transformed infrared spectroscopy (Klajnert et al. 2007b). The phosphorous dendrimers were able to interfere with PrP(185–208) aggregation process by both decreasing the formation of aggregates and by reducing the final amount of amyloid fibrils.

Dendrimers as therapeutic agents and their potential as anti-prion, anti-Alzheimer's, anticoagulant, antidote, anti-inflammatory, and anticancer agents were reviewed (Gajbhiye et al. 2009; Rojo and Delgado 2007; Rojo 2009; Sekowski et al. 2008; Heegaard et al. 2007).

The "magic staff"—from magic bullet to dendrimeric magic forks

Paul Ehrlich proposed the magic bullet concept over a century ago. His idea has slowly turned into reality (Bremer et al. 2009; Heynick 2009; Strasser 2008; Pini et al. 2008). The concept of magic bullet refers to selective killing of target cells by specific drug or antibody. The use of peptide molecules as specific bullets for targeting pathological markers and pathogens has largely been limited by their short half-lives (Pini et al. 2008).

Attempts to develop more effective treatments for diseases which are polygenic in origin, and the most effective medications of which have exceedingly complex pharmacologies (such as schizophrenia and depression) by discovering drugs selective for single molecular targets (that is, "magic bullets") have, not surprisingly, been largely unsuccessful. There is a growing interest in polypharmacy (Bianchi et al. 2009), a concept that has been described as the "magic shotgun", "promiscuous", or "dirty" approaches (Bianchi et al. 2009). The magic shotgun approach targets multiple components of a complex system and represents a substantial departure from the dominant high specificity of "magic bullet". The authors (Roth et al. 2004) designed selectively non-selective drugs ("magic shotguns"), which interact with several molecular targets. These can serve as new and more effective medications for a variety of central nervous system disorders (Roth et al. 2004). Treatment of complex disease processes supports potential benefits of "magic shotgun" over "magic bullet" approaches. The shotgun approach is not meant to imply that with multiple targets, one can increase the chances of modulating the single critical one. Instead, it implies that multiple targets are relevant for seizure pathophysiology and by extension, its pharmacotherapy (Bianchi et al. 2009).

A novel method based on "armed" oligo-branched peptides as tumor targeting drugs was called "magic forks" (Falciani et al. 2009). Branched peptides, such as MAPs (Tam 1988) have been extensively tested to reproduce single epitopes to stimulate the immune system for new vaccine discovery. Pini et al. discovered (Falciani et al. 2007; Pini et al. 2008) that peptides in MAP format acquire

strong resistance to peptidases and proteases. This resistance makes MAPs very stable and thus suitable for drug development. The use of several MAP molecules in different biotechnological applications (antimicrobial and antitoxin peptides, tumor targeting, etc.) was reviewed (Pini et al. 2008). The MAP approach provides branched molecules that do not exist in nature, and are resistant to endogenous proteases and peptidases. Proteolytic resistance of branched multimeric sequences in human plasma and serum was compared to that of the same peptides synthesized as multimeric linear molecules. In linear multimeric sequences, an increase in peptide copies did not increase peptide resistance, whereas multimericity progressively enhanced proteolytic stability of branched multimeric peptides (MAPs) (Falciani et al. 2007; Pini et al. 2005, 2008). Tetra branched MAPs are particularly suitable for in vivo use, and good candidates for the development of peptide based therapeutics. These protease-resistant oligobranched peptides escape difficulties related to use of peptide as drugs. MAPs are very stable toward enzymatic degradation by blood peptidases and they can be conjugated with several functional units. These units can serve for tumor cell imaging or targeting. Conjugation does not interfere with peptide binding to targets. Moreover, branched peptides bind much tighter to the target due to their multivalency. MAPs can play a role of Trojan horses capable to selectively transport chemotherapy drugs into the tumor cells (Falciani et al. 2009).

Recommended literature on dendrimers in general

A number of excellent reviews have been published on *dendrimers* in general (Chabre and Roy 2010; Astruc et al. 2010; Tomalia 2009; Rosen et al. 2009; van Dongen et al. 2009; Svenson 2009; Carlmark et al. 2009; Svenson and Chauhan 2008; Newkome and Shreiner 2008; Chabre and Roy 2008; Roy and Touaibia 2007; Kehat et al. 2007; Balogh 2007; Frauenrath 2005; Boas et al. 2006a, g; Heegaard and Boas 2006; Tomalia 2005a; Svenson and Tomalia 2005; Caminade et al. 2005; Smith et al. 2005; Lockman et al. 2005; Frauenrath 2005; Liang and Frechet 2005); *MAPs* (Scholl et al. 2009; Kehat et al. 2007; Crespo et al. 2005; Tam 2004); *MAGs and glycodendrimers* (Chabre and Roy 2010, 2008; Imberty et al. 2008; Niederhafner et al. 2008a, b; Roy and Touaibia 2007; Li et al. 2007; Touaibia and Roy 2007; Tsvetkov and Nifantiev 2005; Roy 2003; Lundquist and Toone 2002); *carbopeptides* (Roy and Touaibia 2007; Jensen and Brask 2002, 2005); and *their biomedical applications* (Chabre and Roy 2010; Dutta et al. 2010; Tekade et al. 2009b; Gabius 2009; Bharali et al. 2009; Paleos et al. 2009; Labieniec and Watala 2009; Rolland et al. 2009; Svenson and Chauhan 2008; Cheng et al. 2008b; Paleos et al. 2008; Toth et al. 2008; Niederhafner et al. 2008c; Roy and Touaibia 2007; Boas et al. 2006b, c, d; Doores et al. 2006; Heegaard and Boas 2006; Svenson and Tomalia 2005; Dufes et al. 2005).

Conclusions

Peptide and especially glyco- and glycopeptide dendrimers represent the best high-density coding system. Their information potential and chemical diversity are the reason for enormous functional variability, which paves the way for many applications in biological sciences (drug and gene delivery, solubilization effect on drugs, antimicrobial, antiviral, antiprion and anticancer activities, contrast agents for molecular imaging, etc.); material sciences (design of new hydrogels, nanomaterials, and nanotechnologies); catalysis, analytical chemistry, and forensic science. The interplay between dendrimer-, click-, Lego-, and variety of ligation chemistries lead to explosive acceleration of new significant discoveries.

Tissue engineering applications for healing of spine injuries and construction of artificial tissues for cultivation of stem cells can emerge in near future. Self-healing hydrogels with very low abundance of dendrimeric materials can substitute conventional plastics. The nanoscale of dendrimeric catalysts will enable the synthesis of artificial enzymes designed for specific purposes. Combination of small targeting peptides or tumor-associated antigens with dendrimeric cargos will open a possibility for development of tailor-made anticancer synthetic vaccines. Potentially, dendrimers will support treatment of neurodegenerative disorders, such as Alzheimer's disease and prion caused diseases.

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