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Designer peptide dendrimers using click reaction

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ABSTRACT

We designed and synthesized various peptide dendrimers using a 1,3-dipolar cycloaddition (Click) reaction. The dendritic structures reported here include symmetrical, asymmetrical, and cationic dendrimers with triazole, cystine, aromatic, aliphatic, and Lys–Asp dipeptide cores. The high chemoselectivity of the click reaction allowed us to synthesize good yields of high-purity protected and unprotected dendritic structures. Triazole is an excellent peptide bond mimic, which remains hydrolytically stable. Dendrimer **15a** and the core unit **21** gelate in a mixture of organic solvents. We also demonstrated the versatility of the design by synthesizing various carbohydrate-based dendrimers.

1. Introduction

Dendrimers are structurally well-defined molecules with a branched architecture and a large number of functional groups at the surface. They also display multivalent interactions (the cluster effect).[1] Dendrimers have applications in diverse areas including nanobiotechnology and tissue engineering.[2–6] The potential applications of dendrimers have generated intense research interest, although there are challenges involved in their synthesis. All synthetic strategies for dendrimers rely on iterative sequences of reaction steps, which yield geometric increases in the number of surface functionalities. Modular reactions are essential for the chemoselective ligation of dendrons required to synthesize full-sized dendrimers. In order to be effectively used for dendrimer synthesis, these modular reactions must proceed efficiently in sterically hindered environments. Recently, 1,3-dipolar cycloaddition reactions have roused considerable interest among researchers because of their nearly quantitative yield, mild reaction conditions, and broad tolerance towards functional groups.

The thermal 1,3-dipolar cycloaddition reaction between alkyne and azide has been known for over a century and was thoroughly investigated by Rolf Huisgen and Koch in the 1950s.[7] The reaction is thermodynamically favored (ΔH ~55 kcal/mol) but has a high kinetic energy barrier (~26 kcal/mol). The kinetic stability of alkyne and azide make the reaction very slow at room temperature. However, the addition of a Copper (I) catalyst accelerates the reaction rate up to 10^7 times and exclusively yields the 1,4 regioisomer (Fig. 1).[8] These transformations are called 'click' reactions.[9]

The triazole moiety forms a rigid linking unit that mimics the electronic properties of a peptide bond. The distance between R^3 and R^4 (Fig. 2) is 3.9 Å in a peptide bond, but in a triazole structure the distance is approximately 5.0 Å. However, a triazole unit has a higher dipole moment than a peptide, which ensures correct peptide bond mimicry.[10]

The chemoselective ligation of two unprotected peptide fragments to a full-sized protein is a challenging endeavor. Researchers have developed a number of chemical reactions that can be used to overcome this challenge. The native chemical ligation,[11] Staudinger,[12] and thiaproline[13] reactions are elegant and efficient methods of

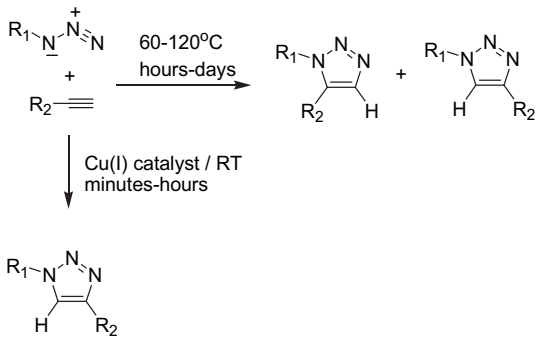

Fig. 1. 1,3-Dipolar cycloaddition reaction between azide and alkyne.

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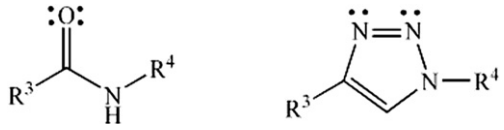


Fig. 2. Comparison between the structure of a peptide bond and a triazole unit.

synthesizing proteins. Development of the expressed protein ligation approach further testifies to the combined efforts of chemists and biologists to solve this problem.[14]

Dendrimers are devoid of secondary structural elements but have compact globular topology. The synthesis of such macromolecular structures, combined with de novo design strategies, may provide protein-like molecules with catalytic and/or other functional properties. However, robust and high-yielding chemical reactions are needed to synthesize protein-like macromolecules effectively. The click reaction is an attractive option for chemoselectively linking two dendrons.[15–17] Recently, we have used the click reaction to synthesize a variety of peptide dendrimers.[18]

There are many examples of dendrimer synthesis dealing with peptide and non-peptide architecture, but very few synthetic strategies are suitable for generating dendrimers with unprotected functionalities.[19] In addition to its high yield and high degree of functional group tolerance, the click reaction is advantageous in that it proceeds without altering other functional groups. We envisioned that this reaction could be used to link various protected and unprotected peptide fragments. Triazole is an excellent peptide bond mimic, while at the same time remaining hydrolytically stable. The synthesis of heteromeric dendritic structures[20] has rarely been reported in the literature despite possible advantages of this architecture over conventional symmetric dendrimers. Asymmetrical dendrimers could be specifically targeted to various biomolecules, making it possible to develop multi-targeting molecular systems.

2. Results and discussion

We have used the high chemoselectivity and bioorthogonality of the click reaction to design various peptide-based designer dendrimers. Amino acids with side chain functionality can be chosen as building blocks for dendrimer synthesis. The protective group chemistry of amino acids is already well established; we therefore chose to synthesize dendrimers based on amino acids. Our strategy was to anchor the alkyne on one dendron and the azide on the other dendron (Fig. 3a). These alkyne- and azide-truncated dendrons were then linked using click reactions to yield dendrimers. In another design strategy, a dialkyne core was chosen and the dendrons, functionalized with azide units, were coupled to yield the dendrimer (Fig. 3b). Alternately, diazido spacers were coupled to dendrons functionalized with alkyne units (Fig. 3c).

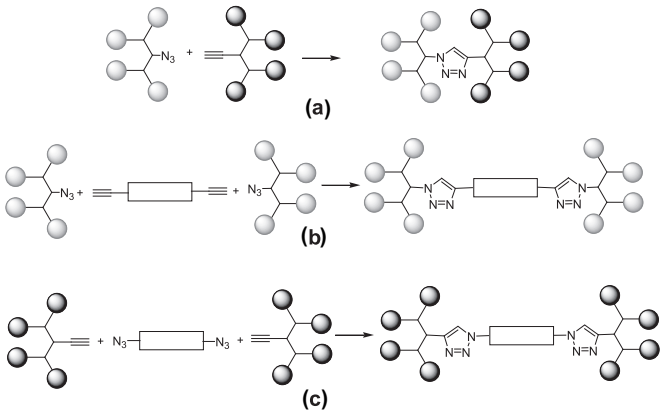


Fig. 3. Basic design principle for the synthesis of peptide dendrimers using click reaction.

With this design strategy in mind, we synthesized alkyne- and azide-truncated peptide dendrons. Aspartic acid (Asp) is an attractive choice for the synthesis of peptide dendrons because the presence of a side chain carboxylic acid group allows it to act as a branching unit in dendrimer synthesis. Azide-truncated Asp-based dendrons **1a** and **1b** were synthesized in good yields following the strategy demonstrated in our earlier reports.[18]

Lysine (Lys) was chosen as the monomer unit to synthesize an alkyne-truncated dendron because it has a side chain amino group that can act as a branching unit. Different generations of alkyne-terminated dendrons (**2a**, **2b**, **3a**, and **3b)** were synthesized from BocLys(Boc)-OH.

With these dendrons (Fig. 4), we synthesized the dendrimers in a convergent fashion using the strategies outlined in Fig. 3. In order to synthesize asymmetrical dendrimers **4a**, **4b**, and **5**, a dendron truncated with an alkyne was reacted with an azide-truncated dendron (Scheme 1). To synthesize a symmetrical dendrimer, dendrons were attached to a symmetrical dialkyne or diazide core (Schemes 2–6).

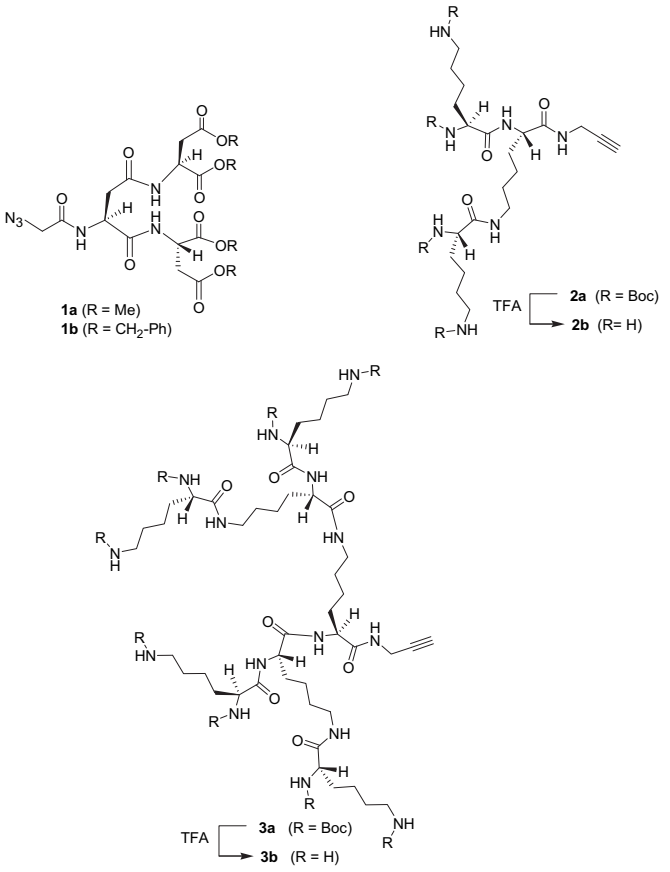
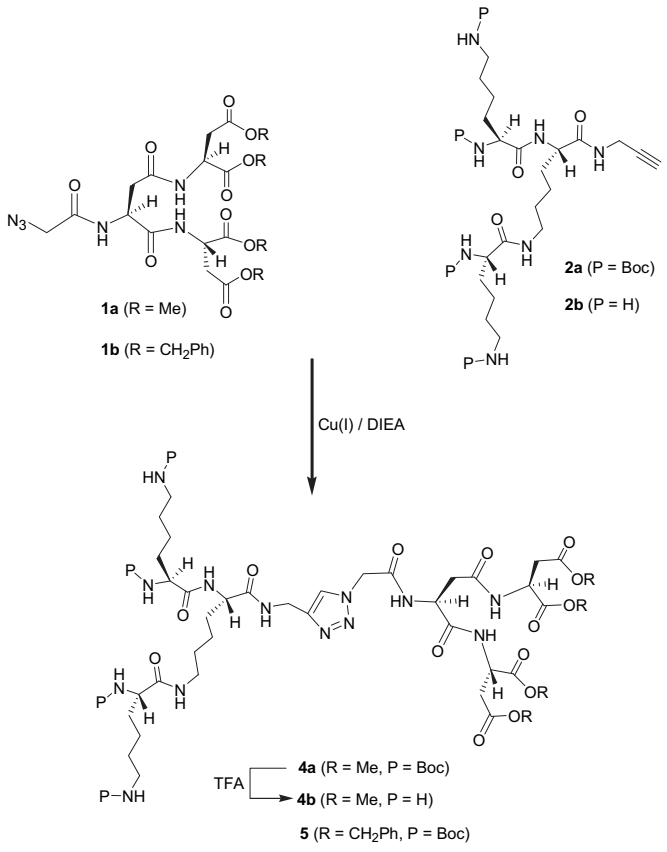


Fig. 4. Structures of various peptide-based dendrons.

In order to synthesize dual-surface dendrimers **4a**, **4b**, and **5**, we linked an aspartic acid-based dendron appended with an azido group at the N-terminus and a lysine-based dendron truncated with an alkyne at the C-terminus (Scheme 1). The reaction of **1a** and **2a** in a Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction produced 1,4-substituted triazole-based dendrimer **4a** in ~68% yield with four Boc protected amines and four carbomethoxy units. The Boc groups were deprotected to generate the cationic dendrimer **4b** in quantitative yield (Scheme 1). Dendrimer **4b** was also synthesized

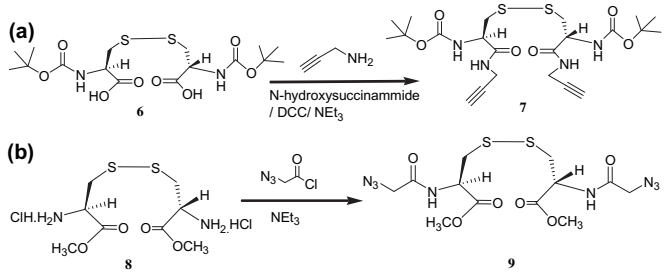


Scheme 1. Synthesis of unsymmetrical dendrimers **4a**, **4b**, and **5.**

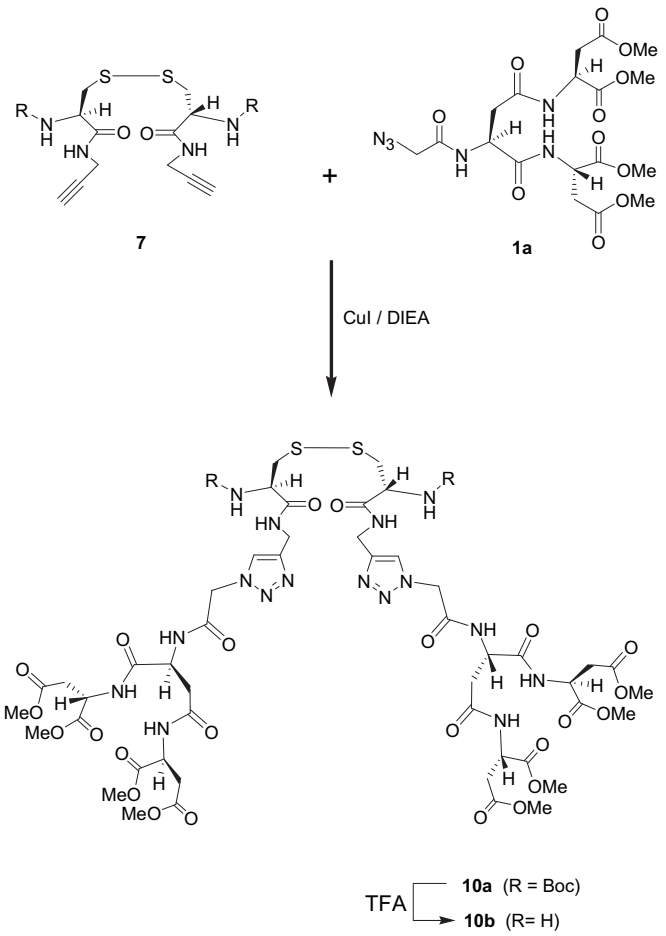
by reacting tetraamine **2b** with **1a**. Similarly, dendrimer **5** was synthesized from benzyl-protected Asp dendron **1b**. Dendrimer **5** was synthesized to demonstrate that different groups can be added to the periphery of the dendritic surface. This robust and flexible dendrimer synthesis, able to generate amino terminals, carboxyl terminals, and a combination of the two, will be of profound interest to scientists working in various disciplines.

Cystine, with two carboxylate groups, can be functionalized with a propargyl amine to produce a dialkyne suitable as a core unit. The dialkyne core **7** was synthesized in 91% yield by coupling Boc-Cystine **6** with propargyl amine in the presence of DCC and *N*-hydroxysuccinimide (Scheme 2). Similarly, the cystine core **9**, carrying two azide functionalities, was synthesized from cystine diOMe·2HCl **8**. The reaction between compound **8** and azidoacetyl chloride generated compound **9**; this is an ideal core unit for a reaction with a Lys-dendron appended with an alkyne unit.

The reaction of Asp dendron **1a** with **7** produced the symmetrical dendrimer **10a** (Scheme 3). Similarly, the reaction of core **9** with dendron **2a** afforded dendrimer **11a** (Scheme 4). Dendrimer **10a** was deprotected with TFA to obtain **10b**, with potential for further synthetic extrapolation. Lysine-based dendron **3a,** on



Scheme 2. Synthesis of cystine-based cores (a) dialkyne core **7** (b) diazide core **9.**

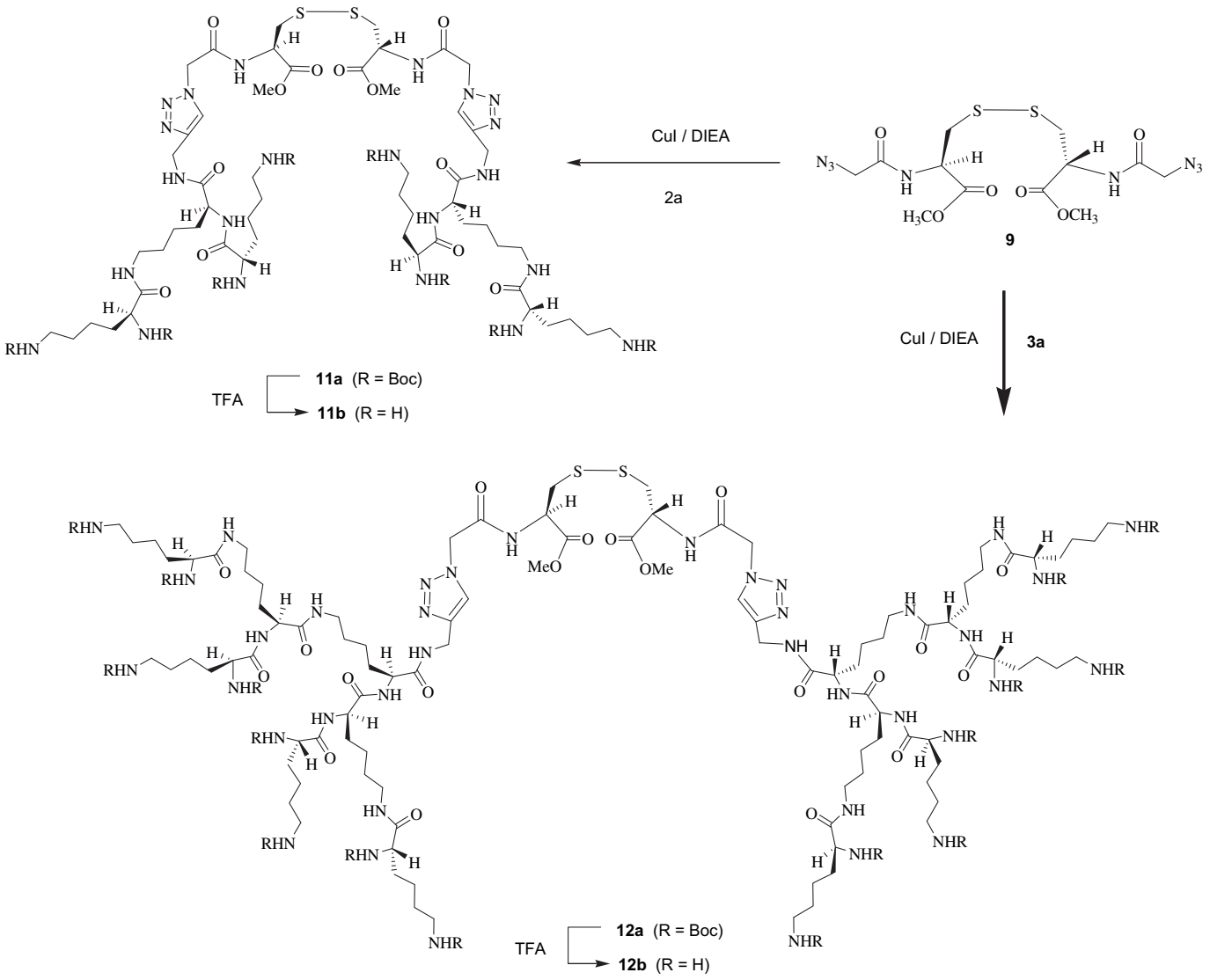


Scheme 3. Synthesis of dendrimers **10a** and **10b.**

reaction with azide core **9**, provided dendrimer **12a**. The yields of these reactions were in the range of 65–92%. The post-reaction workup afforded reasonably pure compounds. Purified dendrimers were obtained by passing the compounds through small silica gel columns. The purity of all the dendrimers synthesized was confirmed by reversed phase analytical HPLC. The ^{1}H NMR spectrum of **11a** showed the presence of a triazole CH, indicating the formation of the desired product. The high resolution mass spectrum (HRMS) of **11a** (calculated mass 2136.1425, measured mass 2136.1436), further confirmed the formation of the proposed dendrimer.

A cystine core can be replaced with a *p*-xylylene or 1,4-diazidobutane. The reaction of an alkyne-terminated dendron (**2a**, **2b**, **3a** or **3b**) with diazides (**13** or **14**) provided dendrimers **15a**, **15b**, **16a**, **16b**, **17a**, **17b**, **18a**, and **18b** (Schemes 5 and 6). This chemoselectivity is an important aspect of the click reaction: it can be used to synthesize anionic and cationic peptide dendrimers in good yields.

It is desirable to have a dendrimeric structure with further possibilities for conjugation to biomolecular systems. In order to demonstrate the versatility of our strategy, we synthesized dendrimers using a Lys–Asp dendron as the central core unit. The dipeptide core **21** was synthesized from BocLys(Boc)-OH **19** and the dialkyne derivative of aspartic acid **20**. This dipeptide core with two alkyne units can be attached to dendrons, producing a dendrimer. The Lys unit with α and ε amino groups can be deprotected for further chemical extrapolation. The Asp-based dendron **1a** was attached via click reaction to the Lys–Asp scaffold **21** to give dendrimer **22** in 70% yield (Scheme 7). The unused Lys Nα and Nε amino handles allow other dendrons to be attached. Moreover, these Nα and Nε amino groups could in principle also be reacted with molecules of biological significance.



Scheme 4. Synthesis of symmetrical dendrimers **11a**, **11b**, **12a**, and **12b.**

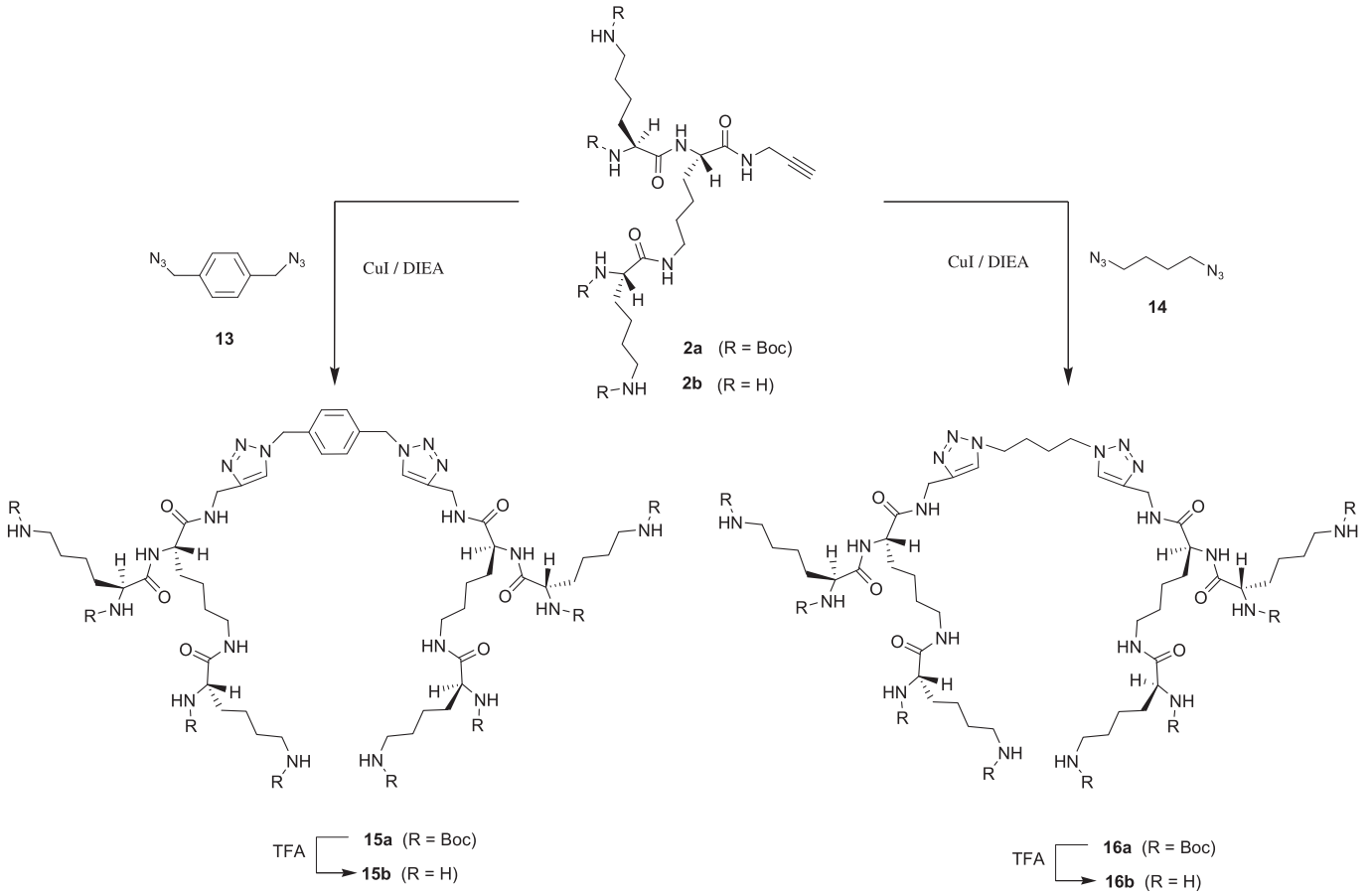
It is noteworthy that the dendrimer **15a** displayed gelling properties in a mixture of chloroform and hexane (1:2). Similarly, compound **21** gelated in a mixture of ethyl acetate and hexane (1:5). These gels were examined using scanning electron microscope (SEM) and were found to have a fibrous morphology (Fig. 5). The fibers are having widths mostly in the range of 0.37–0.63 μm. These observations suggest that the triazole-based dendrimers aggregate in solution and can be further fine-tuned to form organo- or hydrogels. Gels have potential uses in pharmaceutical, cosmetics, new material development, and as sensors.[21] Therefore, the gelation properties exhibited by compounds **15a** and **21** open up new avenues for further exploration.

Peptide–carbohydrate conjugates are becoming immensely relevant because of their biological relevance.[1,22] Carbohydrate dendrimers have significant biological activity, for example, enhanced binding to cell surface receptors due to their multivalent nature.[23] The multivalent effect is not fully understood and therefore requires an in-depth study. Dendrimers are excellent candidates to address the question of multivalency via the creation of diverse classes of dendrimers. We have demonstrated that our strategy is a versatile method for synthesizing dendrimers with various carbohydrate moieties on the periphery. The azide-functionalized carbohydrate unit was synthesized from the pentaacetylated carbohydrate derivatives. Reaction of peracetylated glucosyl acetate or galactosyl acetate with tin tetrachloride and trimethylsilyl azide provided the corresponding azides in good yield.[24] The azide-functionalized carbohydrate was reacted with cores, such as **23** and **26** to yield dendrimers **25a**,**b** and **27a**,**b** (Schemes 8 and 9).

The topological and electronic similarities of a triazole ring to a peptide bond, the large dipole, and the presence of two nitrogens as hydrogen bond acceptors, make triazole an excellent peptide bond surrogate.[25] A triazole unit may impart rigidity and protease stability to the dendritic structure. Therefore, incorporation of a non-hydrolysable heterocyclic moiety in the structure of a dendrimer may provide added advantages.

3. Conclusions

In conclusion, we have demonstrated a simple and elegant method to synthesize designer peptide dendrimers with complex architecture using click chemistry. A series of symmetric and asymmetric dendrimers were synthesized based on the 1,3-dipolar cycloaddition reaction. The yield of synthesis was good, ranging from 65 to 92%. The functional group-tolerant nature of the click reaction allowed us to link dendrons with unprotected



Scheme 5. Synthesis of symmetrical lysine dendrimers **15a**, **15b**, **16a**, and **16b**.

functionalities. The synthesis of dendrimers with a dipeptide (Lys–Asp) core is another feature of our design; the additional lysine can carry units important for biological recognition. We also found that dendrimer **15a** and the core **21** are good organogelators. In summary, we have demonstrated a good design principle and synthesis of various peptide dendrimers with good yield and purity.

4. Experimental section

4.1. Preparation of lysine-based dendrons

4.1.1. Preparation of propargylamide of bis Boc-lysine. To an ice cold solution of BocLys(Boc)-OH (2.10 g, 6.07 mmol) in 90 ml of dry dichloromethane was added *N*-hydroxysuccinimide (0.766 g, 6.65 mmol), DCC (1.377 g, 6.67 mmol), and stirred for 10 min. Afterwards, propargyl amine (0.456 ml, 6.65 mmol) and triethylamine (0.926 ml, 6.65 mmol) were added and stirred overnight. Filtered the reaction mixture, washed the filtrate with 0.2 N H_2SO_4, water, and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 2.20 g BocLys(Boc)-propargylamide.

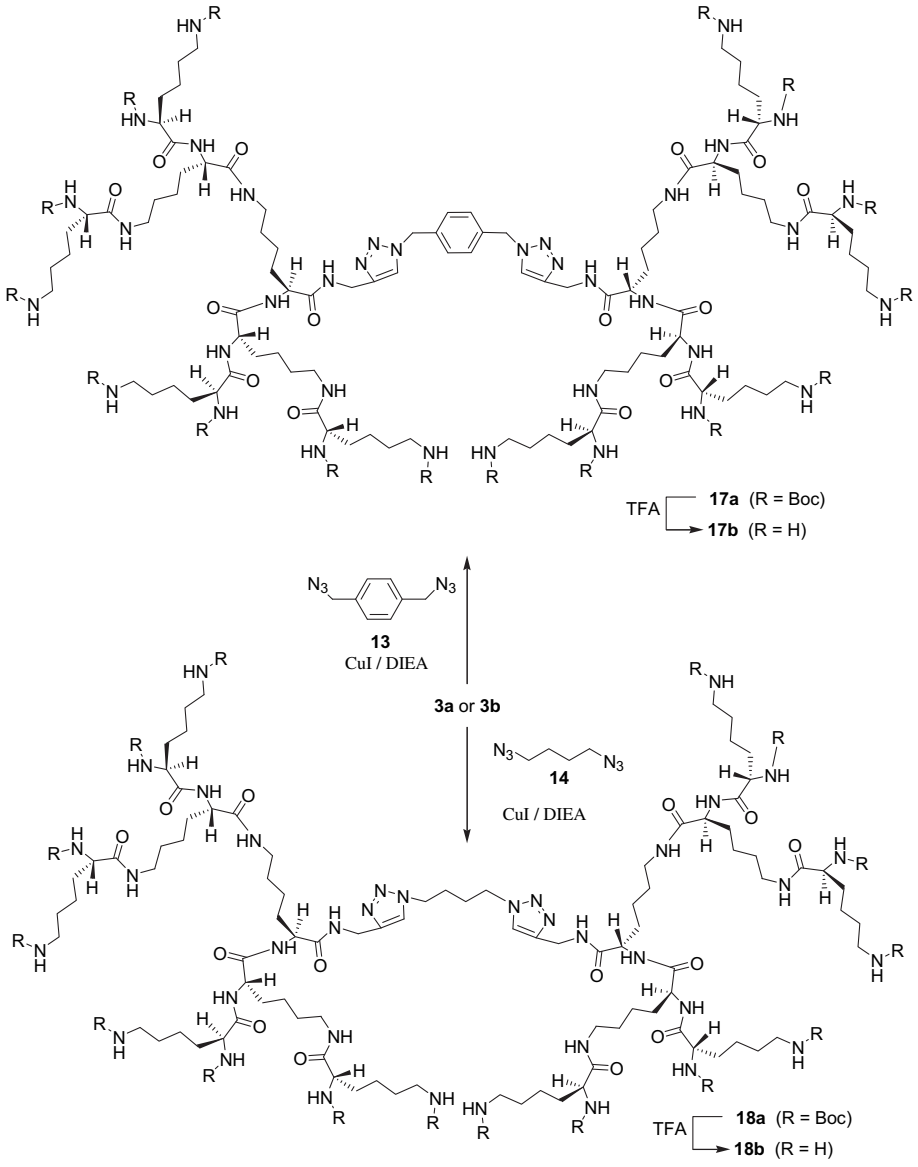
Yield: 94%; $[\alpha]_D^{23.4}$ −1.8 (*c* 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38–1.70 (s+m, 24H), 2.20 (t, *J*=2.4 Hz, 1H), 3.10 (m, 2H), 4.04 (m, 3H), 4.56 (br s, 1H), 5.03 (br d, 1H), 6.46 (br s, 1H); IR (KBr) 3333, 2977, 2935, 2866, 1687, 1524, 1453, 1376, 1249, 1171 cm^{-1}; HRMS calcd for $C_{19}H_{34}N_3O_5$ *m*/*z* 384.2498, found *m*/*z* 384.2500.

*4.1.2. Preparation of Lys dendron **2a**.* To an ice cold solution of BocLys (Boc)-OH (1.982 g, 5.73 mmol) in 80 ml of dry dichloromethane was added *N*-hydroxysuccinimide (0.723 g, 6.28 mmol), DCC (1.297 g, 6.28 mmol) and stirred for 10 min. Afterwards, propargylamide of lysine (0.525 g, 2.87 mmol) and triethylamine (0.875 ml, 6.29 mmol) were added and stirred overnight. Filtered, washed the filtrate with 0.2 N H_2SO_4, water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to yield 2.10 g of the compound **2a**.

Yield: 87%; $[\alpha]_D^{24.5}$ −45.0 (*c* 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.37–1.85 (s+m, 54H), 2.25 (s, 1H), 3.10 (m, 6H), 4.02 (s, 2H), 4.09–4.45 (m, 3H), 4.70–5.00 (m, 2H), 5.60 (br s, 1H), 5.95 (br s, 1H), 7.14 (br d, 2H), 7.43 (br s, 1H); IR (KBr) 3340, 2932, 2862, 1691, 1651, 1527, 1450, 1372, 1249, 1169 cm^{-1}; HRMS calcd for $C_{41}H_{73}N_7O_{11}Na$ *m*/*z* 862.5266, found *m*/*z* 862.5244.

*4.1.3. Preparation of Lys dendron **3a**.* To an ice cold and well-stirred solution of BocLys(Boc)-OH (1.447 g, 4.18 mmol) in 70 ml of dry dichloromethane was added *N*-hydroxysuccinimide (0.481 g, 4.18 mmol), DCC (0.862 g, 4.18 mmol) and stirred for 10 min. To this was added propargylamide of Lys(Lys)Lys-OH (0.408 g, 0.929 mmol), triethylamine (0.581 ml, 4.18 mmol) and stirred overnight. Filtered, washed the filtrate with 0.2 N H_2SO_4, water and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to yield 1.22 g of the compound **3a**.

Yield: 75%; $[\alpha]_D^{24}$ +28.66 (*c* 0.15, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15–2.05 (br m, 114H), 2.20 (s, 1H), 2.95–3.60 (br m, 14H), 3.90–4.60 (br m, 9H), 4.72–5.25 (br m, 4H), 5.52–6.18 (br m, 4H), 6.68–7.20 (br m, 2H), 7.32–7.90 (br m, 5H); IR (KBr) 3325, 3082, 2976, 2934, 2861, 2247, 1693, 1649, 1527, 1452, 1392, 1367, 1249, 1171 cm^{-1}; HRMS calcd for $C_{85}H_{153}N_{15}O_{23}Na$ *m*/*z* 1775.1161, found *m*/*z* 1775.1142.



Scheme 6. Synthesis of symmetrical Lys dendrimers based on aromatic and aliphatic cores.

*4.1.4. Preparation of Lys dendron **3b**.* To an ice-cooled solution of **3a** (0.160 g, 0.091 mmol) in dry CH_2Cl_2 (1 ml) was added TFA (3 ml) and stirred at RT for 2 h. The reaction mixture was subjected to vacuum to afford 0.080 g of **3b**.

Yield: 92%; ^{1}H NMR (D_2O, 300 MHz) δ 0.95–1.80 (m, 42H), 2.26 (s, 1H), 2.67 (br s, 8H), 2.87 (br s, 6H), 3.52–3.79 (m, 6H), 3.81–4.05 (m, 3H); IR (KBr) 3430, 2935, 1664, 1436, 1383, 1197, 1138 cm^{-1}; HRMS calcd for $C_{45}H_{90}N_{15}O_7$ *m/z* 952.7148, found *m/z* 952.7155.

4.2. Preparation of azidoacetyl chloride

To an ice-cooled solution of sodium azide (0.936 g, 14.4 mmol) in 8 ml water was added bromoacetic acid (1.0 g, 7.19 mmol) and stirred for 24 h. The reaction mixture was acidified with HCl to pH ~2 and extracted with diethyl ether (3×100 ml). The organic layer was dried over Na_2SO_4, and evaporated in vacuo to afford 0.710 g of the product.

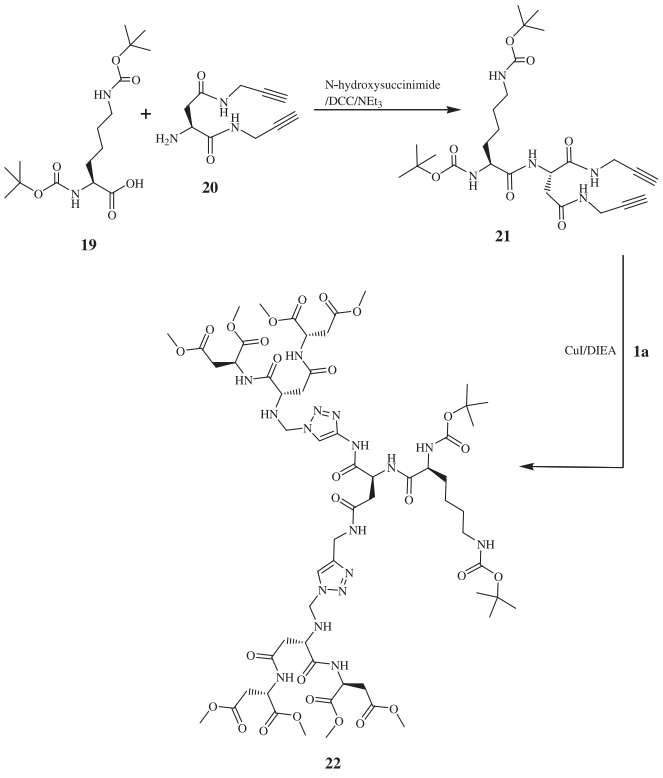
Yield: 97%; ^{1}H NMR (D_2O, 300 MHz) δ 3.72 (s, 2H); ^{13}C NMR (D_2O, 75 MHz) δ 52.58, 176.08; IR (KBr) 3449, 2950, 2128, 1612, 1409, 1299 cm^{-1}.

To an ice cold solution of azidoacetic acid (0.404 g, 4.0 mmol) in 5 ml of dry dichloromethane was added 2 drops of dry DMF and oxalyl chloride (0.412 ml, 4.8 mmol) and stirred for 24 h and reaction mixture was evaporated to yield 0.450 g azidoacetyl chloride.

Yield: 94%; IR (KBr) 3375, 2928, 2109, 1835, 1746, 1642, 1419, 1284 cm^{-1}.

4.3. Preparation of *Z*-Asp(AspdiOMe)AspdiOMe

To a well-stirred and ice-cooled solution of *Z*-Aspartic acid (1.50 g, 5.62 mmol) in 70 ml dry CH_2Cl_2 was added *N*-hydroxysuucinimide (1.62 g, 14.07 mmol), DCC (2.90 g, 14.05 mmol), Asp·diOMe·HCl (2.77 g, 14.02 mmol) containing triethylamine (1.95 ml, 14.04 mmol). After stirring for 24 h at room temperature, the reaction mixture was filtered. The residue was washed with CH_2Cl_2 (4×20 ml) and the combined filtrates were washed sequentially with 2 N H_2SO_4, water, and 5% aqueous $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and evaporated in vacuo to afford 2.90 g of the product.



Scheme 7. Synthesis of dendrimer with Lys–Asp as the central core unit.

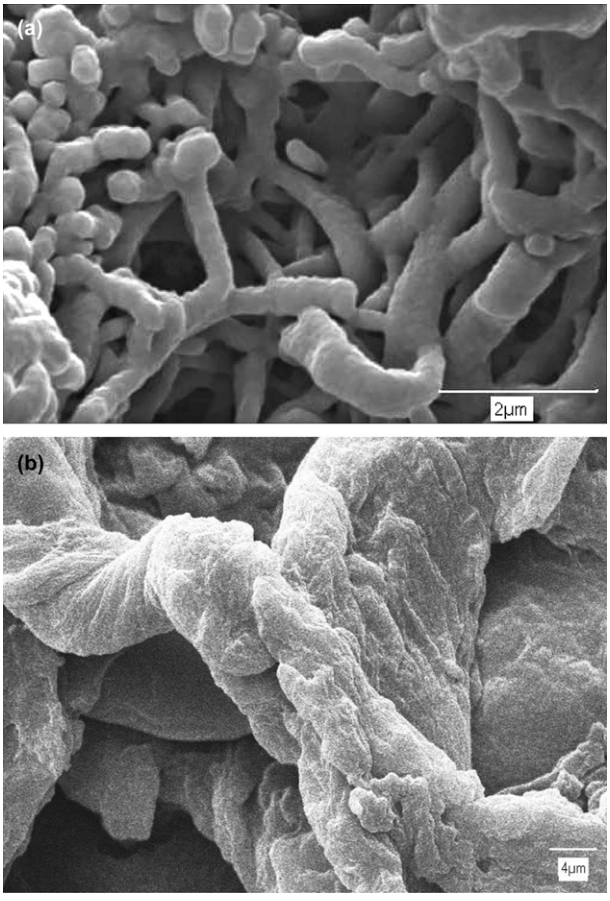
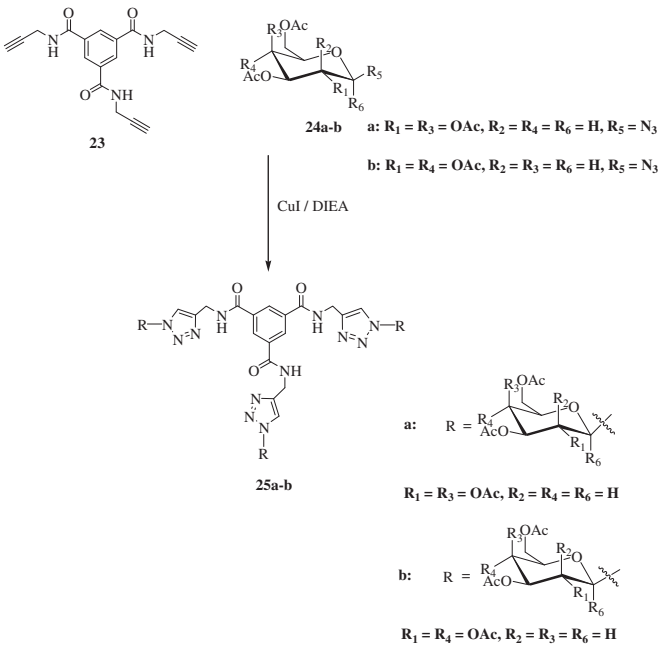
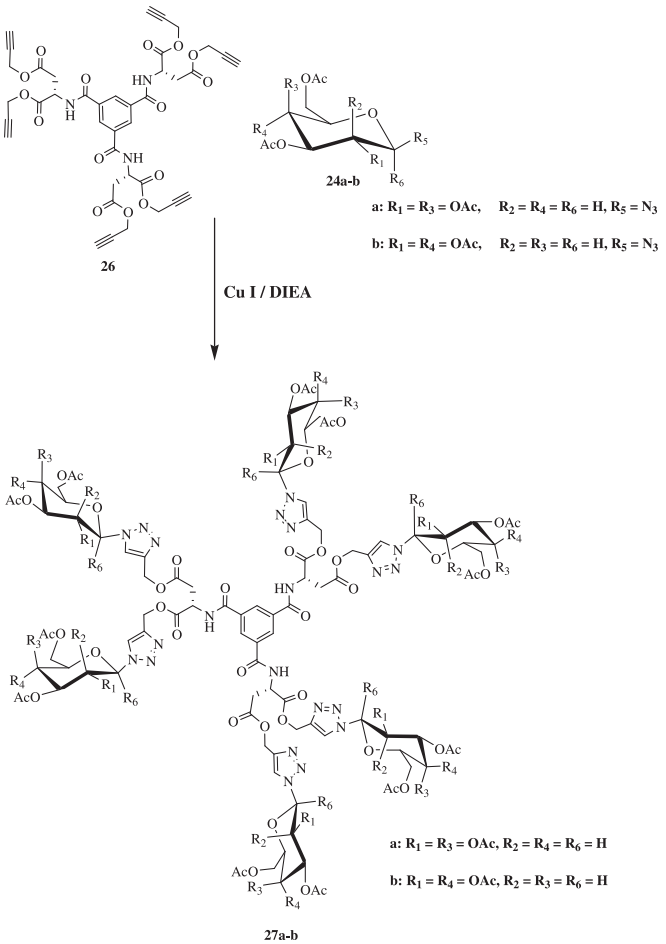


Fig. 5. SEM images of gels from (a) **21** (Ethyl acetate/hexane (1:5)) (b) **15a** (Chloroform/hexane (1:2)).



Scheme 8. Synthesis of various carbohydrate-based dendrimers on a trialkyne core.



Scheme 9. Synthesis of carbohydrate dendrimers on a hexaalkyne core.

Yield: 93%; $[\alpha]_D^{24.4}$ −8.5 (*c* 0.26, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.63–3.10 (m, 6H), 3.72 (s, 6H), 3.77 (s, 6H), 4.63 (m, 1H), 4.85 (m, 2H), 5.16 (s, 2H), 6.28 (d, *J*=6.6 Hz, 1H), 6.73 (d, *J*=8.1 Hz, 1H), 7.38 (s, 5H), 7.94 (br d, 1H); IR (KBr) 3307, 3084, 2955, 2854, 1739, 1698, 1648, 1545, 1438, 1366, 1300 cm^{-1}; HRMS calcd for $C_{24}H_{31}N_3O_{12}Na$ *m*/*z* 576.1805, found *m*/*z* 576.1793.

4.4. Preparation of Asp dendron 1a

An ice-cooled solution of *Z*-Asp-(Asp·diOMe)Asp·diOMe (0.221 g, 0.4 mmol) in 10 ml of dry MeOH was admixed with 10% Pd/C, (peptide/catalyst 1:0.5 w/w), and H_2 was bubbled through the reaction mixture for 1.5 h. After completion of the reaction (TLC), the solution was filtered, and the filtrate was evaporated. The residue obtained was dissolved in dry dichloromethane. The solution was cooled in an ice-bath and triethylamine (0.056 ml, 0.4 mmol) was added, followed by the slow addition of azidoacetyl chloride **5** (0.048 g, 0.4 mmol) over a period of 0.5 h. The reaction mixture was left stirred at room temperature for 12 h. The solvent was removed in vacuo, the solid obtained was dissolved in ethyl acetate (50 ml), washed, with 2 N H_2SO_4, water, and 5% aqueous $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, evaporated and purified by silica gel column chromatography using EtOAc/hexane to give 0.144 g of **1a**.

Yield: 72%; $[\alpha]_D^{22.6}$ +54.7 (*c* 0.10, $CHCl_3$); mp 135–136 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.60–3.20 (m, 6H), 3.70 (s, 6H), 3.75 (s, 3H), 3.76 (s, 3H), 4.02 (s, 2H), 4.82 (m, 3H), 6.85 (d, *J*=7.4 Hz, 1H), 7.65 (d, *J*=7.5 Hz, 1H), 7.87 (d, *J*=7.0 Hz, 1H); IR (KBr) 3310, 2959, 2854, 2111, 1741, 1666, 1644, 1536, 1436, 1373, 1281 cm^{-1}; HRMS calcd for $C_{18}H_{27}N_6O_{11}$ *m*/*z* 503.1738, found *m*/*z* 503.1732.

4.5. Preparation of unsymmetrical dendrimer 4a

To an ice-cooled solution of **2a** (0.151 g, 0.18 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.031 ml, 0.18 mmol), N_3-Asp-(Asp·diOMe)Asp·diOMe **1a** (0.090 g, 0.18 mmol) and CuI (0.004 g, 0.021 mmol). The reaction mixture was stirred under nitrogen atmosphere for 17 h. The reaction mixture was evaporated, the solid thus obtained was washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution and finally with water. The residue obtained was dried and crystallized from a mixture of chloroform and methanol to give 0.163 g dendrimer **4a**.

Yield: 68%; $[\alpha]_D^{24.6}$ −9.4 (*c* 0.22, MeOH); mp 158–160 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.10–1.70 (s+m, 54H), 2.71–3.20 (m, 12H), 3.60 (s, 12H), 3.81 (br s, 2H), 4.31 (m, 3H), 4.63 (m, 3H) 5.09 (br s, 2H), 6.74 (m, 2H), 6.90 (d, *J*=7.8 Hz, 1H), 7.74 (br s, 2H), 7.80 (s, 1H), 8.44 (m, 3H), 8.58 (d, *J*=7.8 Hz, 2H); IR (KBr) 3438, 2932, 1753, 1643, 1533, 1448, 1370, 1246, 1171 cm^{-1}; HRMS calcd for $C_{59}H_{99}N_{13}O_{22}Na$ *m*/*z* 1364.6925, found *m*/*z* 1364.6909.

4.6. Preparation of unsymmetrical dendrimer 4b

To an ice-cooled solution of **4a** (0.025 g, 0.0186 mmol) in 1 ml of dry CH_2Cl_2 was added 3 ml of TFA. The solution was left stirred for 2 h and the reaction was monitored by TLC. The reaction mixture was subjected to high vacuum to yield 0.016 g of the dendrimer **4b**.

Yield: 91%; ^{1}H NMR (D_2O, 300 MHz) δ 1.00–2.00 (m, 18H), 2.55–3.20 (m, 12H), 3.62 (m, 12H), 3.80–4.00 (m, 3H), 4.00–4.20 (m, 2H), 4.67 (m, 2H, merged with D_2O residual peak), 5.10–5.30 (m, 3H), 7.83 (s, 1H); HRMS calcd for $C_{39}H_{68}N_{13}O_{14}$ *m*/*z* 942.5009, found *m*/*z* 942.5039.

4.7. Preparation of Dendrimer 5

4.7.1. Preparation of Boc-Asp(OBzl)OBzl. To a solution of Boc-Asp-OH (3.5 g, 15.01 mmol) in 50 mL dry dichloromethane was added *N*-hydroxysuccinimide (3.7 g, 32.14 mmol), DCC (6.6 g, 32.14 mmol), benzyl alcohol (3.3 ml, 31.7 mmol), and triethylamine (4.4 ml, 32.14 mmol). The reaction mixture was kept at 0 °C and stirred for 12 h. Evaporated the reaction mixture, dissolved in ethyl acetate, and washed with 0.2 N H_2SO_4 and water. The organic layer was dried over Na_2SO_4 and evaporated to obtain 4.58 g of Boc-Asp(OBzl)OBzl.

Yield 73.9%; $[\alpha]_D^{25.8}$ +4.90 (*c* 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H), 2.88 (dd, *J*=17.4 Hz, 4.8 Hz, 1H), 3.07 (dd, *J*=17.4 Hz, 4.8 Hz, 1H), 4.65 (m, 1H), 5.08 (s, 2H), 5.14 (s, 2H), 5.53 (d, *J*=7.8 Hz, 1H), 7.27–7.42 (m, 10H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.5, 37.1, 50.3, 67.0, 67.6, 80.4, 128.6, 128.8, 135.6, 155.6, 171.1; IR (KBr) 3433, 3032, 2975, 1742 (br), 1504, 1457, 1355, 1165 cm^{-1}; HRMS calcd for $C_{23}H_{27}NO_6Na$ *m*/*z* 436.1736, found *m*/*z* 436.1738.

4.7.2. Preparation of Boc-Asp(Asp(OBzl)OBzl)-Asp(OBzl)OBzl. To an ice-cooled and well-stirred solution of Boc-Asp-OH (1.02 g, 4.4 mmol) in 50 mL of dry dichloromethane was added *N*-hydroxysuccinimide (1.12 g, 9.6 mmol) and DCC (2.0 g, 9.6 mmol). The reaction mixture was stirred for 10 min, added Asp(OBzl)OBzl (3.0 g, 9.6 mmol) and triethylamine (1.3 ml, 9.6 mmol). The reaction mixture was stirred for 12 h at room temperature and filtered. Filtrate was washed sequentially with aqueous 0.2 N H_2SO_4, $NaHCO_3$, and water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to get 2.48 g of Boc-Asp(Asp(OBzl)OBzl)-Asp(OBzl)OBzl.

Yield: 67.3%; $[\alpha]_D^{23}$ +33.16 (*c* 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 9H), 2.51 (m, 1H), 2.84 (dd, *J*=17.0, 4.6 Hz, 2H), 2.98 (dd, *J*=17.1, 4.8 Hz, 2H), 4.48 (m, 1H), 4.82 (m, 2H), 5.03 (s, 2H), 5.05 (s, 2H), 5.08 (s, 2H), 5.11 (s, 2H), 5.97 (d, *J*=6.0 Hz, 1H), 6.75 (d, *J*=8.1 Hz, 1H), 7.20–7.40 (br m, 20H), 7.60 (d, *J*=7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.2, 36.1, 37.4, 48.8, 50.9, 66.8, 67.5, 80.2, 128.3, 128.5, 135.1, 135.3, 155.5, 170.2, 170.6, 170.9; IR (KBr) 3319, 3064, 3034, 2975, 1738, 1696, 1650, 1529, 1455, 1388, 1361, 1284, 1169 cm^{-1}; HRMS calcd for $C_{45}H_{49}N_3O_{12}Na$ *m*/*z* 846.3214, found *m*/*z* 846.3247.

4.8. Preparation of azide-truncated dendron 1b

To an ice-cooled solution of Asp(Asp(OBzl)OBzl)-Asp(OBzl)OBzl (0.263 g, 0.36 mmol) in 30 ml of dry dichlorormethane was added triethylamine (0.25 ml, 1.8 mmol) and followed by addition of azidoacetyl chloride (0.065 g, 0.54 mmol). The reaction mixture was stirred for 12 h and washed sequentially with 2 N H_2SO_4, $NaHCO_3$ solution, and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to get 0.170 g of dendron.

Yield: 58%; $[\alpha]_D^{21.4}$ +18.1 (*c* 0.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.47–2.62 (m, 1H), 2.70–3.10 (m, 5H), 3.91 (s, 2H), 4.71–4.92 (m, 3H), 4.99–5.17 (m, 8H), 6.83 (d, *J*=7.8 Hz, 1H), 7.21–7.38 (m, 20H), 7.65 (d, *J*=7.2 Hz, 1H), 7.81 (d, *J*=7.2 Hz, 1H); IR (KBr) 3428, 2925, 2855, 2108, 1738, 1651, 1540, 1390, 1182 cm^{-1}; ESI MS m/z 807 ($M+H^+$).

4.9. Preparation of dendrimer 5

To an ice-cooled solution of **2a** (0.042 g, 0.050 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.008 ml, 0.05 mmol), **1b** (0.040 g, 0.05 mmol) and CuI (0.0093 g, 0.005 mmol). The reaction mixture was stirred under nitrogen atmosphere for 17 h. The reaction mixture was evaporated, the solid thus obtained was washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution and finally with water. The residue obtained was dried and crystallized from a mixture of chloroform and methanol to give 0.0524 g of dendrimer **5**.

Yield: 64%; $[\alpha]_D^{25.1}$ −8.7 (*c* 0.24, $CHCl_3$+MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20–1.80 (m, 54H), 2.49–3.30 (m, 12H), 3.90–4.29 (br m, 2H), 4.29–4.40 (br m, 2H), 4.51 (br m, 2H), 4.70–4.90 (m, 4H), 4.95–5.21 (m, 8H), 5.40–5.85 (br d, 3H), 5.92 (br s, 2H), 6.70–7.10 (br d, 2H), 7.20–7.50 (br m, 20H), 7.50–7.70 (br m, 2H), 7.77–7.90

(br m, 2H); IR (KBr) 3311, 3069, 2928, 2859, 1688, 1646, 1529, 1456, 1387, 1248, 1171 cm^{-1}; ESI MS found *m*/*z* 1668 ($M+Na^+$).

4.10. Preparation of cystine core 7

To an ice cold solution of bis Boc-Cystine (0.8 g, 1.82 mmol) in 90 ml of dry dichloromethane was added *N*-hydroxysuccinimide (0.460 g, 3.99 mmol) and DCC (0.824 g, 3.99 mmol). The reaction mixture was stirred for 10 min, afterwards propargyl amine (0.274 ml, 3.99 mmol) and triethylamine (0.56 ml, 4.02 mmol) were added and stirred overnight. Filtered the reaction mixture, washed the filtrate with 0.2 N H_2SO_4, water, and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.850 g of the compound **7**.

Yield: 91%; $[\alpha]_D^{24.9}$ +57.7 (*c* 0.38, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.48 (s, 18H), 2.18 (s, 2H), 2.91 (m, 4H), 4.25 (m, 4H), 4.93 (br s, 2H), 5.55 (d, *J*=9.3 Hz, 2H), 8.06 (br s, 2H); IR (KBr) 3307, 2978, 2928, 2853, 1660, 1525, 1446, 1372, 1251, 1168 cm^{-1}; HRMS calcd for $C_{22}H_{34}N_4O_6S_2Na$ *m*/*z* 537.1817, found *m*/*z*.

4.11. Preparation of cystine core 9

To an ice-cooled solution of Cystine·diOMe·2HCl (0.674 g, 1.976 mmol) in 40 ml dry dichloromethane was added dry triethylamine (1.10 ml, 7.91 mmol) followed by the slow addition of azidoacetyl chloride (0.473 g, 3.96 mmol) over a period of 0.5 h and stirred at room temperature for 12 h. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate (50 ml), washed with 2 N H_2SO_4, water, and 5% aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.670 g of the compound **9**.

Yield: 78%; $[\alpha]_D^{23.4}$ +58.7 (*c* 0.23, $CHCl_3$); ^1H NMR (D_2O, 300 MHz) δ 3.06 (m, 2H), 3.33 (m, 2H), 3.76 (s, 6H), 4.06 (s, 4H), 4.83 (m, 2H); IR (KBr) 3331, 3069, 2996, 2953, 2102, 1731, 1658, 1546, 1442, 1394, 1326, 1240 cm^{-1}; HRMS calcd for $C_{12}H_{18}N_8O_6S_2K$ *m*/*z* 473.0428, found *m*/*z* 473.0439.

4.12. Preparation of symmetrical Asp dendrimer 10a

To an ice-cooled solution of dialkyne **7** (0.52 g, 0.101 mmol) in 20 ml of dry acetonitrile under nitrogen was added diisopropylethylamine (0.037 ml, 0.215 mmol), N_3-Asp-(Asp·diOMe) Asp·diOMe **1a** (0.101 g, 0.201 mmol), and CuI (0.004 g, 0.021 mmol). The reaction mixture was stirred under N_2 atmosphere for 17 h and the solvent was evaporated. The solid thus obtained was dissolved in chloroform, washed with 2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.10 g of the dendrimer **10a**.

Yield: 65%; $[\alpha]_D^{25.5}$ +23.4 (*c* 0.28, $CHCl_3$); ^1H NMR (DMSO-d_6, 300 MHz) δ 1.35 (s, 18H), 2.65–2.90 (m, 12H), 3.00–3.25 (m, 4H), 3.60 (s, 24H), 4.00–5.00 (m, 6H), 4.61 (s, 6H), 5.07 (s, 4H), 7.02 (s, 2H) 7.81 (s, 2H), 8.37–8.70 (m, 8H); IR (KBr) 3321, 2958, 1739, 1654, 1537, 1444, 1370, 1231, 1173 cm^{-1}; HRMS calcd for $C_{58}H_{86}N_{16}O_{28}S_2Na$ *m*/*z* 1541.5137, found *m*/*z* 1541.5132.

4.13. Preparation of symmetrical Asp dendrimer 10b

To an ice-cooled solution of **10a** (0.050 g, 0.033 mmol) in 1 ml of dry CH_2Cl_2 was added 2 ml of TFA. The solution was left stirred at 0 °C for 2 h and the reaction mixture was subjected to high vacuum to yield 0.040 g of the dendrimer **10b**.

Yield: 92%; ^1H NMR (DMSO-d_6, 300 MHz) δ 1.23 (m, 4H), 2.62–2.75 (m, 12H), 2.80–3.08 (m, 4H), 3.62 (s, 24H), 4.07 (m, 2H), 4.36 (m, 2H), 4.47–4.80 (m, 8H), 5.11 (s, 4H), 7.93 (s, 2H), 8.22–8.72 (m, 6H), 9.13 (s, 2H); ^1H NMR (D_2O, 300 MHz) δ 2.55–2.90 (m, 12H), 2.95–3.18 (m, 4H), 3.51 (s, 6H), 3.56 (s, 6H), 3.59 (s, 6H), 3.61 (s, 6H), 4.15–4.55 (m, 8H), 5.16 (s, 8H), 7.99 (s, 2H); IR (KBr) 3259, 3071, 2962, 1738, 1669, 1541, 1440, 1375, 1177 cm^{-1}; HRMS calcd for $C_{48}H_{70}N_{16}O_{24}S_2Na$ *m*/*z* 1341.4088, found *m*/*z* 1341.4077.

4.14. Preparation of symmetrical Lys dendrimer 11a

To an ice-cooled solution of **2a** (0.240 g, 0.286 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.05 ml, 0.29 mmol), **9** (0.62 g, 0.142 mmol), and CuI (0.006 g, 0.031 mmol). The reaction mixture was stirred under N_2 atmosphere for 18 h. The reaction mixture was evaporated, dissolved in ethyl acetate. Washed the filtrate with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.440 g of the dendrimer **11a**.

Yield: 73%; $[\alpha]_D^{20.9}$ −16.8 (*c* 0.26, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.27–1.82 (m, 108H), 2.87–3.46 (m, 12H), 3.68 (s, 6H), 3.87–4.67 (m, 12H), 4.78 (m, 6H), 5.08 (m, 4H), 5.37–6.00 (m, 4H), 6.70–7.17 (m, 4H), 7.35–7.80 (m, 6H), 7.94 (s, 2H); IR (KBr) 3325, 2975, 2935, 1690 (br), 1523, 1451, 1367, 1248, 1171 cm^{-1}; HRMS calcd for $C_{94}H_{164}N_{22}O_{28}S_2Na$ *m*/*z* 2136.1425, found *m*/*z* 2136.1436.

4.15. Preparation of symmetrical Lys dendrimer 11b

To an ice-cooled solution of **11a** (0.030 g, 0.0142 mmol) in 0.5 ml of dry CH_2Cl_2 was added 2 ml of TFA. The solution was left stirred for 2 h and subjected to high vacuum to yield dendrimer **11b**.

Yield: 91%; IR (KBr) 3403, 2927, 1744, 1682, 1542, 1439, 1180 cm^{-1}; HRMS calcd for $C_{54}H_{101}N_{22}O_{12}S_2$ *m*/*z* 1313.7411, found *m*/*z* 1313.7411.

4.16. Preparation of symmetrical Lys dendrimer 12a

To an ice-cooled solution of Lys_7-alkyne **3a** (0.30 g, 0.171 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.03 ml, 0.174 mmol), **9** (0.0373 g, 0.086 mmol), and CuI (0.006 g, 0.031 mmol). The reaction mixture was stirred under N_2 atmosphere for 17 h and solvent was evaporated. The solid thus obtained was dissolved in ethyl acetate, washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield dendrimer **12a**.

Yield: 67%; $[\alpha]_D^{21.5}$ −24.1 (*c* 0.17, MeOH); ^1H NMR (DMSO-d_6, 300 MHz) δ 0.90–1.60 (m, 228H), 2.60–2.70 (m, 28H), 3.43 (m, 10H), 3.63 (m, 10H), 3.87–4.20 (m, 12H), 4.41 (m, 2H), 4.89 (m, 4H), 6.08 (m, 10H), 6.28 (m, 4H), 7.15–7.42 (br m, 10H), 7.59 (br d, 2H), 7.83 (br d, 2H), 8.49 (br d, 2H); IR (KBr) 3299, 2930, 2861, 1691, 1646, 1527, 1453, 1367, 1248, 1171 cm^{-1}; HRMS calcd for $C_{182}H_{324}N_{38}O_{52}S_2Na$ *m*/*z* 3961.3216, found *m*/*z* 3961.3030.

4.17. Preparation of symmetrical Lys dendrimer 12b

To an ice-cooled solution of **12a** (0.040 g, 0.010 mmol) in 0.5 ml of dry CH_2Cl_2 was added 3 ml of TFA. The solution was left stirred for 2 h and subjected to high vacuum to yield dendrimer **12b**.

Yield: 92%; ^1H NMR (D_2O, 300 MHz) δ 1.07–1.90 (m, 84H), 2.75–3.20 (m, 28H), 3.62 (s, 6H), 3.70–4.22 (m, 18H), 4.25–4.45 (m, 6H), 5.19 (m, 4H), 7.80 (s, 2H); IR (KBr) 3433, 3089, 2929, 1677, 1544, 1435, 1201, 1135 cm^{-1}; HRMS calcd for $C_{102}H_{197}N_{38}O_{20}S_2$ *m*/*z* 2338.5008, found *m*/*z* 2338.5006.

4.18. Preparation of symmetrical dendrimer 15a

To an ice-cooled solution of **2a** (0.420 g, 0.50 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.14 ml, 0.81 mmol), *p*-xylylene diazide (**13**) (0.047 g, 0.25 mmol), and CuI (0.010 g, 0.053 mmol). The reaction mixture was stirred under N_2 atmosphere for 18 h, evaporated the solvent, and dissolved in ethyl acetate. It was then washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.377 g of the dendrimer **15a**.

Yield: 81%; $[\alpha]_D^{23.8}$ −13.5 (*c* 0.57, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.10–1.90 (s+m, 108H), 2.70–2.95 (br s, 12H), 3.80–4.12 (br m, 4H), 4.12–4.45 (m, 6H), 4.65 (br s, 2H), 5.0 (br s, 2H), 5.15–5.40 (m, 4H), 5.72 (br s, 4H), 6.95–7.25 (m, 8H), 7.36 (s, 2H), 7.50 (s, 2H); IR (KBr) 3326, 2931, 2858, 1700, 1659, 1530, 1452, 1367, 1249, 1171 cm^{-1}; HRMS calcd for $C_{90}H_{154}N_{20}O_{22}Na$ *m*/*z* 1890.1444, found *m*/*z* 1890.1463.

4.19. Preparation of symmetrical dendrimer 15b

To an ice-cooled solution of **15a** (0.101 g, 0.054 mmol) in 0.5 ml of dry CH_2Cl_2 was added 2 ml of TFA. The solution was left stirred for 2 h and subjected to high vacuum to yield 0.057 g of the dendrimer **15b**.

Yield: 99%; ^{1}H NMR (D_2O, 300 MHz) δ 1.00–1.85 (br m, 36H), 2.25–3.25 (br m, 12H), 3.72–3.90 (m, 6H), 4.11 (s, 2H), 4.20–4.45 (m, 4H), 5.48 (s, 2H), 7.10–7.30 (br s, 4H), 7.85 (s, 2H); IR (KBr) 3419, 2928, 1674, 1548, 1431, 1198 cm^{-1}; HRMS calcd for $C_{50}H_{91}N_{20}O_6$ *m*/*z* 1067.7430, found *m*/*z* 1067.7409.

4.20. Preparation of symmetrical dendrimer 16a

To an ice-cooled solution of **2a** (0.420 g, 0.50 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.14 ml, 0.81 mmol), butyl diazide (**14**) (0.035 g, 0.25 mmol), and CuI (0.010 g, 0.053 mmol). The reaction mixture was stirred under N_2 atmosphere for 18 h. The reaction mixture was evaporated and dissolved in ethyl acetate. It was then washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.354 g of the dendrimer **16a**.

Yield: 78%; $[\alpha]_D^{26.3}$ −17.4 (*c* 0.38, MeOH); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.15–1.81 (s+m, 112H), 2.82–3.12 (br m, 12H), 3.19 (s, 4H), 3.78–3.92 (m, 4H), 4.18–4.41 (m, 6H), 6.65–6.78 (m, 6H), 6.83–6.95 (br d, 2H), 7.70–7.80 (br m, 4H), 7.86 (br s, 2H), 8.38 (br m, 2H); IR (KBr) 3328, 3082, 2976, 2935, 2866, 1694, 1650, 1522, 1453, 1367, 1248, 1171 cm^{-1}; HRMS calcd for $C_{86}H_{154}N_{20}O_{22}Na$ *m*/*z* 1842.1444, found *m*/*z* 1842.1384.

4.21. Preparation of symmetrical dendrimer 16b

To an ice-cooled solution of **16a** (0.101 g, 0.056 mmol) in 0.5 ml of dry CH_2Cl_2 was added 2 ml of TFA. The solution was left stirred for 2 h and subjected to high vacuum to yield 0.056 g of the dendrimer **16b**.

Yield: 98%; ^{1}H NMR (D_2O, 300 MHz) δ 1.15–2.02 (br m, 40H), 2.25–3.25 (br m, 12H), 3.68–4.21 (m, 8H), 4.27–4.50 (m, 6H), 7.80–7.91 (m, 2H); IR (KBr) 3079, 2938, 1674, 1546, 1432, 1198 cm^{-1}; HRMS calcd for $C_{46}H_{91}N_{20}O_6$ *m*/*z* 1019.7430, found *m*/*z* 1019.7403.

4.22. Preparation of symmetrical dendrimer 17a

To an ice-cooled solution of **3a** (0.430 g, 0.25 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.071 ml, 0.41 mmol), **13** (0.024 g, 0.128 mmol), and CuI (0.005 g, 0.026 mmol). The reaction mixture was stirred under N_2 atmosphere for 17 h and solvent was evaporated. The solid thus obtained was dissolved in ethyl acetate, washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.321 g of the dendrimer **17a**.

Yield: 68%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.00–1.80 (m, 228H), 2.85–3.15 (m, 28H), 3.80–4.00 (m, 18H), 4.12–4.40 (br m, 10H), 5.64 (br s, 4H), 6.62–7.05 (br m, 12H), 7.65–8.05 (br m, 12H), 8.36 (br s, 2H); IR (KBr) 3329, 2930, 2855, 1693, 1632, 1575, 1532, 1448, 1367, 1247, 1171 cm^{-1}; HRMS calcd for $C_{178}H_{314}N_{36}O_{46}Na$ *m*/*z* 3715.3236, found *m*/*z* 3715.3226.

4.23. Preparation of symmetrical dendrimer 17b

To an ice-cooled solution of **17a** (0.101 g, 0.027 mmol) in 0.5 ml of dry CH_2Cl_2 was added 3 ml of TFA. The solution was left stirred for 2 h and subjected to high vacuum to yield 0.057 g of the dendrimer **17b**.

Yield: 100%; ^{1}H NMR (D_2O, 300 MHz) δ 1.00–1.85 (m, 84H), 2.80–3.10 (m, 28H), 3.62–3.92 (m, 6H), 4.02–4.20 (m, 10H), 4.26–4.50 (br m, 2H), 5.47 (s, 4H), 7.19 (s, 4H), 7.89 (br s, 2H); IR (KBr) 3435, 2575, 1779, 1670, 1340, 1171 cm^{-1}; HRMS calcd for $C_{98}H_{187}N_{36}O_{14}$ *m*/*z* 2092.5028, found *m*/*z* 2092.4950.

4.24. Preparation of symmetrical dendrimer 18a

To an ice-cooled solution of **3a** (0.438 g, 0.25 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.071 ml, 0.41 mmol), **14** (0.0175 g, 0.125 mmol), and CuI (0.005 g, 0.026 mmol). The reaction mixture was stirred under N_2 atmosphere for 17 h and solvent was evaporated. The solid thus obtained was dissolved in ethyl acetate, washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.388 g of the dendrimer **18a**.

Yield: 85%; $[\alpha]_D^{26.2}$ −11.2 (*c* 0.25, MeOH); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.13–1.82 (m, 232H), 2.80–3.10 (m, 28H), 3.86 (br m, 12H), 4.11–4.41 (m, 12H), 5.55–5.69 (br m, 4H), 6.60–6.90 (m, 12H), 7.60–8.01 (br m, 12H), 8.32 (br s, 2H); IR (KBr) 3332, 2931, 2856, 1693, 1639, 1529, 1450, 1393, 1367, 1247, 1171 cm^{-1}; HRMS calcd for $C_{174}H_{314}N_{36}O_{46}Na$ *m*/*z* 3667.3236, found *m*/*z* 3667.3271.

4.25. Preparation of symmetrical dendrimer 18b

To an ice-cooled solution of **18a** (0.040 g, 0.011 mmol) in 0.5 ml of dry CH_2Cl_2 was added 3 ml of TFA. The solution was left stirred for 2 h and subjected to high vacuum to yield 0.022 g of the dendrimer **18b**.

Yield: 100%; ^{1}H NMR (D_2O, 300 MHz) δ 1.15–1.90 (m, 88H), 2.85–3.20 (m, 28H), 3.80–4.00 (m, 6H), 4.08–4.40 (m, 6H), 4.70 (m, 10H) 8.36 (br s, 2H); IR (KBr) 3324, 3071, 2929, 2854, 1700, 1626, 1579, 1444, 1313, 1182 cm^{-1}; HRMS calcd for $C_{94}H_{187}N_{36}O_{14}$ *m*/*z* 2044.5028, found *m*/*z* 2044.5069.

4.26. Preparation of Lys–Asp core 21

*4.26.1. Preparation of **20**.* To an ice cold solution of Boc-Asp-OH (0.8 g, 3.43 mmol) in 90 ml of dry dichloromethane was added *N*-hydroxysuccinimide (0.869 g, 7.55 mmol) and DCC (1.557 g, 7.55 mmol) and stirred for 10 min. To this propargyl amine (0.517 ml, 7.54 mmol) and triethylamine (1.050 ml, 7.55 mmol) were added and stirred overnight. Filtered, washed the filtrate with 0.2 N H_2SO_4, water, and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4,

filtered, and evaporated to yield 0.90 g of the compound Boc-Asp-dialkyne.

Yield: 85%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.45 (m, 9H), 2.23 (m, 2H), 2.57 (m, 1H), 2.89 (m, 1H), 4.04 (m, 4H), 4.48 (m, 1H), 6.14 (br s, 1H), 6.39 (br s, 1H), 7.19 (br s, 1H); IR (KBr) 3312, 2932, 2127, 1694, 1643, 1532, 1443, 1332, 1276, 1167 cm^{-1}; HRMS calcd for $C_{15}H_{21}N_3O_4Na$ m/z 330.1430, found m/z 330.1429.

To an ice-cooled solution of Boc-Asp-dialkyne (0.700 g, 2.28 mmol) in 6 ml of dry CH_2Cl_2 was added 3 ml of TFA. The solution was left stirred for 2 h and completion of the reaction was monitored by TLC. The reaction mixture was subjected to high vacuum to yield 0.472 g of the NH_2-Asp-dialkyne **20**. It was used as such without further purification.

*4.26.2. Preparation of **21**.* BocLys(Boc)-OH (0.788 g, 2.28 mmol) was dissolved in 90 ml of dry dichloromethane, cooled to 0 °C. To this ice cold solution was added *N*-hydroxysuccinimide (0.262 g, 2.27 mmol), DCC (0.469 g, 2.27 mmol), and stirred for 10 min. Afterwards NH_2-Asp-dialkyne **20** (0.472 g, 2.28 mmol) and triethylamine (1.268 ml, 9.11 mmol) were added. The reaction mixture was stirred overnight and filtered. Washed the filtrate with 0.2 N H_2SO_4, water, and finally with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield compound **21**.

Yield: 80%; $[\alpha]_D^{23.9}$ +1.8 (c 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.35–2.00 (s+m, 24H), 2.20 (m, 2H), 2.55 (m, 1H), 2.95 (m, 1H), 3.12 (m, 2H), 4.01 (m, 4H), 4.73 (br s, 2H), 5.61 (br s, 1H), 6.68 (br s, 1H), 7.27 (br s, 1H), 7.73 (br s, 1H), 8.04 (br s, 1H); IR (KBr) 3289, 2977, 2933, 2110, 1689, 1647, 1529, 1447, 1367, 1250, 1172 cm^{-1}; HRMS calcd for $C_{26}H_{41}N_5O_7Na$ m/z 558.2904, found m/z 558.2904.

4.27. Preparation of Asp dendrimer 22 with Lys–Asp as the central core

To an ice-cooled solution of **21** (0.050 g, 0.093 mmol) in 20 ml of dry acetonitrile under nitrogen was added diisopropylethylamine (0.016 ml, 0.093 mmol), N_3-Asp-(Asp·diOMe)Asp·diOMe **1a** (0.094 g, 0.187 mmol), and CuI (0.004 g, 0.021 mmol). The reaction mixture was stirred under N_2 atmosphere for 18 h. The reaction mixture was evaporated, dissolved in chloroform, and filtered. Washed the filtrate with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 0.10 g of the dendrimer **22**.

Yield: 70%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.10–1.60 (s+m, 24H), 2.60–2.93 (m, 16H), 3.61 (s, 24H), 3.82 (m, 2H), 4.29 (m, 4H), 4.48–4.72 (m, 6H), 5.07 (m, 4H), 6.71 (br s, 1H), 6.94 (br s, 1H), 7.75–7.90 (m, 2H), 7.97–8.20 (m, 2H), 8.31–8.60 (m, 7H); IR (KBr) 3290, 3081, 2951, 1739, 1648, 1541, 1442, 1368, 1236, 1173 cm^{-1}; HRMS calcd for $C_{62}H_{93}N_{17}O_{29}Na$ m/z 1562.6223, found m/z 1562.6230.

4.28. Preparation of carbohydrate dendrimers

*4.28.1. Synthesis of dendrimers **25a**–**b**.* To an ice-cooled and well-stirred solution of trialkyne **23** (0.032 g, 0.10 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.06 ml, 0.35 mmol), azido sugar (**24a** or **24b)** (0.131 g, 0.35 mmol), and CuI (0.007 g, 0.035 mmol). The reaction mixture was stirred under N_2 atmosphere for 17 h and solvent was evaporated. The solid thus obtained was dissolved in ethyl acetate, washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield the dendrimer.

*4.28.1.1. Dendrimer **25a**.* Yield: 87%; $[\alpha]_D^{27.1}$ −16.3 (c 0.08, $CHCl_3$); mp 148–150 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.79 (s, 9H), 2.02 (br s, 18H), 2.22 (s, 9H), 4.17 (d, J=5.4 Hz, 6H), 4.33 (t, J=6.3 Hz, 3H), 4.74 (br m, 6H), 5.30 (d, J=10.2 Hz, 3H), 5.56 (br s, 3H), 5.64 (t, J=9.75 Hz, 3H), 5.96 (d, J=9.3 Hz, 3H), 8.02 (s, 3H), 8.12 (br s, 3H), 8.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.2, 20.5, 20.67, 20.70, 35.5, 61.1, 66.8, 68.0, 70.8, 73.9, 86.2, 121.6, 128.7, 134.8, 145.4, 166.1, 169.3, 169.9, 170.1, 170.3; IR (KBr) 3390, 2929, 1750, 1660, 1534, 1430, 1373, 1224, 1056 cm^{-1}; HRMS calcd for $C_{60}H_{72}N_{12}O_{30}Na$ m/z 1463.4375, found 1463.4369.

*4.28.1.2. Dendrimer **25b**.* Yield: 90%; $[\alpha]_D^{24.3}$ −31.53 (c 0.33, $CHCl_3$); mp 132–134 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.82 (s, 9H), 1.97 (s, 9H), 2.01 (s, 9H), 2.07 (s, 9H), 4.18 (br d, J=11.1 Hz, 3H), 4.24–4.34 (br m, 6H), 4.74 (br m, 6H), 5.32 (t, J=9.1 Hz, 3H), 5.56 (t, J=9.3 Hz, 3H), 5.73 (t, J=9.1 Hz, 3H), 6.19 (d, J=9.0 Hz, 3H), 8.33 (s, 3H), 8.35 (s, 3H); IR (KBr) 3418, 2925, 2858, 1748, 1652, 1533, 1463, 1423, 1379, 1324, 1226 cm^{-1}; HRMS calcd for $C_{60}H_{72}N_{12}O_{30}Na$ m/z 1463.4375, found 1463.4375.

*4.28.2. Synthesis of dendrimers **27a**,**b**.* To an ice-cooled solution of hexaalkyne **26** (0.05 g, 0.064 mmol) in 20 ml of dry acetonitrile under nitrogen atmosphere was added diisopropylethylamine (0.076 ml, 0.446 mmol), azido sugar (**24a** or **24b)** (0.167 g, 0.446 mmol), and CuI (0.01 g, 0.045 mmol). The reaction mixture was stirred under N_2 atmosphere for 17 h and solvent was evaporated. The solid thus obtained was dissolved in ethyl acetate, washed with 0.2 N H_2SO_4, water, NH_4Cl/NH_4OH (9:1) solution, and finally with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to yield the dendrimer.

*4.28.2.1. Dendrimer **27a**.* Yield: 80%; $[\alpha]_D^{26.1}$ −3.3 (c 0.18, $CHCl_3$); mp 138–140 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.84 (s, 18H), 2.01 (m, 18H), 2.03 (m, 18H), 2.23 (s, 18H), 3.10 (m, 6H), 4.17 (m, 12H), 4.30 (m, 6H), 5.12 (m, 3H), 5.15–5.45 (br m, 18H), 5.55 (m, 12H), 5.92 (d, J=8.1 Hz, 6H), 7.97 (m, 9H), 8.41 (d, J=6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.2, 20.5, 20.7, 36.1, 49.6, 57.8, 58.5, 61.1, 61.2, 66.8, 67.9, 68.0, 70.7, 73.8, 73.9, 86.0, 86.1, 122.7, 123.1, 129.5, 134.4, 142.8, 165.7, 165.8, 169.2, 169.9, 170.1, 170.13, 170.2, 170.3, 170.4; IR (KBr) 3475, 2932, 1752, 1668, 1531, 1375, 1227, 1058 cm^{-1}; HRMS calcd for $C_{123}H_{147}N_{21}O_{69}Na$ m/z 3044.8537, found 3044.8397.

*4.28.2.2. Dendrimer **27b**.* Yield: 80%; $[\alpha]_D^{26.6}$ −29.2 (c 0.11, $CHCl_3$); mp 140–142 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.75–1.92 (m, 18H), 1.97–2.12 (m, 54H), 3.08 (m, 6H), 4.00–4.22 (m, 12H), 4.24–4.38 (br m, 6H), 5.05–5.38 (br m, 21H), 5.45 (t, J=9.3 Hz, 6H), 5.51–5.62 (m, 6H), 5.96 (m, 6H), 7.90 (m, 3H), 7.90–8.15 (m, 6H), 8.37 (d, J=8.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.1, 20.5, 21.0, 36.0, 49.5, 57.8, 58.6, 60.4, 61.6, 67.7, 70.2, 72.6, 75.0, 85.6, 122.8, 123.4, 129.4, 134.4, 142.7, 142.9, 165.6, 168.9, 169.4, 170.0, 170.3, 170.5; IR (KBr) 3403, 2942, 1752, 1667, 1531, 1447, 1376, 1227, 1042 cm^{-1}; HRMS calcd for $C_{123}H_{147}N_{21}O_{69}Na$ m/z 3044.8537, found 3044.8423.

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Supplementary data

^{1}H NMR, ^{13}C NMR, and HRMS of all new compounds. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.01.023.

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