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Recognition of Peptides by Cyclodextrin Trimers

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The efficient synthesis of a new class of cyclodextrin trimers has been carried out by using a click chemistry strategy. The cyclodextrin trimers were subsequently investigated for molecular recognition of peptides with aromatic side chains. Binding affinities for the self-assembly of different peptides to cyclodextrin trimers were determined by using real-time bimolecular interaction analyses with plasmon surface resonance. Peptides were prepared and immobilized on the sensor surface. The association constants were obtained by titration with different solutions of the cyclodextrin trimers and they were in the range of 10^3 M^{-1}. The selectivity of molecular recognition of nonapeptides favored cyclodextrin trimers over unmodified cyclodextrin.

Introduction

For chemists interested in the design and synthesis of molecular receptors that are able to express their recognition features towards a range of different compounds, large structurally constrained ring molecules are an excellent tool.[1] Exploiting noncovalent bonding interactions, synthetic macrocyclic substances, such as crown ethers,[2] calixarenes,[3,4] and cyclophanes,[5] are excellent molecular receptors. These designed receptors are inspired by nature's own large-sized ring compounds, cyclic peptides and peptoids,[6] and carbohydrates (cyclodextrins).[7] Since they, like other carbohydrates, are abundant and readily produced, they have become popular building blocks in receptor molecules.

A distinct drawback in the use of cyclodextrin building blocks is their traditional difficult and low yielding modification, requiring specialist competence. Because cyclodextrins are cyclic oligosaccharides, consisting of 6–8 D-glucopyranose units linked by α-1,4-glycosidic bonds, they have 18–24 hydroxy groups and it is clear that selective modification is nontrivial. On the other hand, it is a very attractive feature that cyclodextrins are water soluble and are known to form thermodynamically stable inclusion complexes with a range of smaller organic compounds in aqueous solution.[8] Cyclodextrins in general show molecular recognitionof aromatic moieties, and specific recognition can be displayed by either α- or β-cyclodextrin, depending on the size of the aromatic species.

DNA and RNA are biopolymers that, through their structure and sequence-selective recognition of complimentary strands, can store and replicate information. Nucleic acids are unique both in structure and properties. They use special base pairing as the tool for interstrand binding and can undergo replication like no other molecule because one strand can act as template for the synthesis of its complementary strand. However, perhaps the properties of DNA can be emulated. Closely related analogues of nucleic acids can indeed copy their properties of sequence selective binding; herein, we wish to go one step further and look at the binding of cyclodextrin oligomers to a peptide strand of aromatic amino acids. Previously, cyclodextrin oligomers have been shown to exhibit characteristic binding specificities of aromatic moieties.[9]

The objective of the present study was to investigate strandlike binding of aromatic functionalities by using trimeric cyclodextrin-based hosts. It was envisaged that a linear compound with phenyl and naphthyl substituents might bind well to a linear assembly of cyclodextrins (Figure 1). For synthetic convenience, a peptide was chosen as the linear attachment site for the aromatic groups. (For previous work on recognition of peptides, see ref.[10]) Since different aromatic groups bind with different affinity to cyclodextrins, selectivity between different peptides is also possible. It is known that α- and β-cyclodextrin display different binding efficiencies for phenyl and naphthyl groups, with the larger β-cyclodextrin binding the naphthyl group stronger than the α-cyclodextrin. While the pK_D of a phenyl group is 2–3 for both α- and β-cyclodextrin, it is 3 and 5, respectively, for the dissociation of the naphthyl group from the cyclodextrin.[8]

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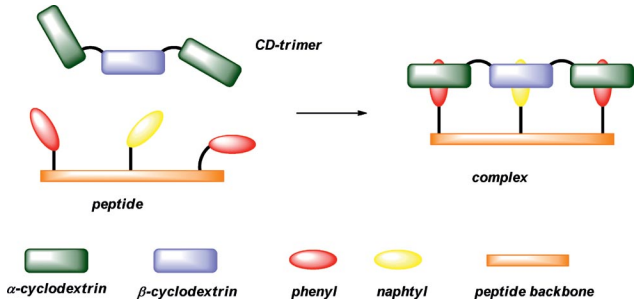


Figure 1. A trimer constructed from cyclodextrins binding a peptide with three aromatic side chains. The β-cyclodextrin preferably binds naphthyl groups.

Results and Discussion

The host–guest complex was analyzed by modeling to establish the optimal spacing between aromatic functionalities to design peptides that could interact with the cyclodextrin trimers. The cyclodextrin oligomers were synthesized by a click chemistry strategy[11,12] from building blocks readily accessible by standard synthetic procedures used in our group. Experimental validation of the calculated observations was conducted by determining binding affinities for the self-assembly of peptides to the cyclodextrin trimers.

Synthesis of Cyclodextrin Building Blocks

The cyclodextrin building blocks intended for preparing the trimers were synthesized as described in Scheme 1. First, the monomer building blocks were prepared: diiodides **1α** and **1β** (Scheme 1) were obtained by perbenzylation of α- and β-cyclodextrin, respectively, using sodium hydride and benzyl bromide in dimethyl sulfoxide,[13] followed by selective debenzylation of the 6^A-, 6^D-positions with diisobutylaluminum hydride,[14] and finally conversion of the 6^A-, 6^D-alcohols to iodides **1α** and **1β**.[15]

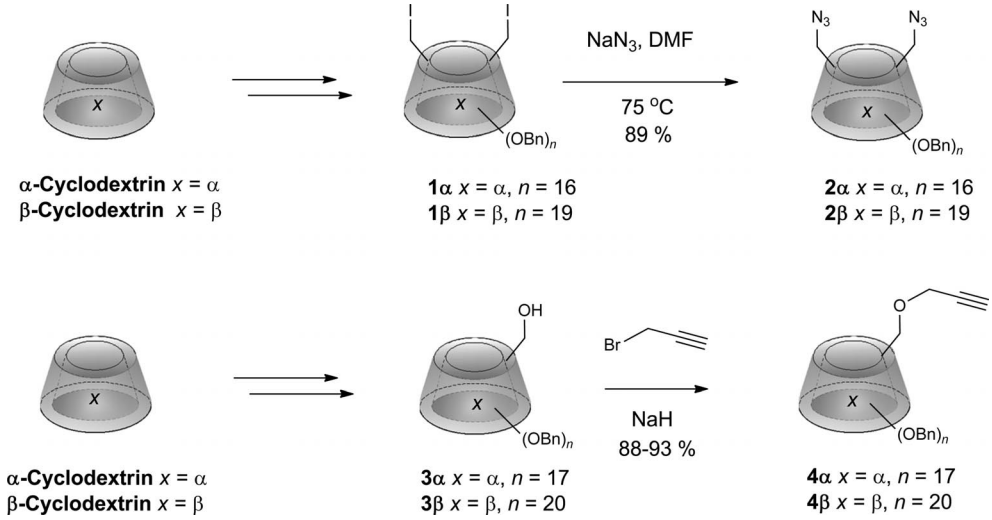
The nucleophilic substitution[16] of **1α** and **1β** to the azides **2α** and **2β** proceeded in excellent yields (Scheme 1). The substitution was carried at 75 °C in dimethyl formamide overnight, affording 80–95% yield after flash chromatography. Notably, the reaction could not be monitored by TLC because compounds **1** and **2** have identical R_f values.

The modification of the 6^A-alcohols **3α** and **3β** into the corresponding propargyl ethers **4α** and **4β**, respectively, was conducted as described in Scheme 1. Treatment of the alcohol with sodium hydride in dimethyl formamide at 0 °C, and subsequent addition of freshly distilled propargyl bromide resulted in full conversion after 4 h to the propargyl ether **4** in 88–93% after flash chromatography.

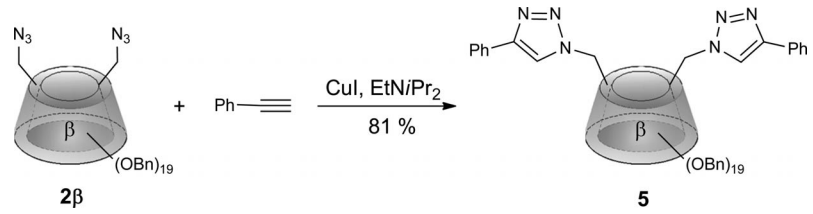
Model Experiments for the Click Reaction

To investigate the viability of the click reaction, we first carried out some model experiments with simpler coupling partners. While examples of successful application of click chemistry to cyclodextrins have been reported,[17,18] these have involved unprotected cyclodextrins for which the usual "click protocol" with water and *tert*-butyl alcohol was employed. Since the benzylated cyclodextrin derivatives **2** and **4** were very poorly soluble in water, a protocol utilizing copper(I) iodide in an organic solvent with an organic base was necessary. Of the different nonaqueous protocols that have been employed for click reactions in the literature,[19,20,21,22] the procedure by Hotha and Kashyap,[19] with the addition of ethyl acetate as a co-solvent, was the successful method.

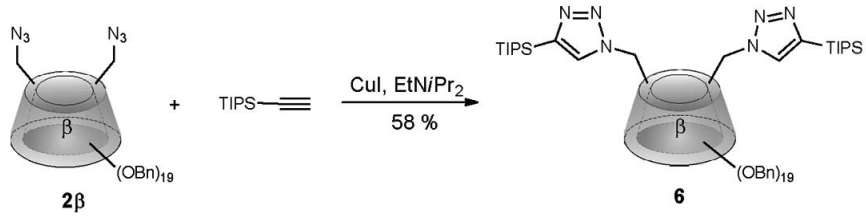
First, we treated **2β** with phenylacetylene in the presence of CuI and diisopropylethylamine (DIPEA) in MeCN/EtOAc. This reaction gave, as expected, only one product **5** in 81% yield after 3 d at 40 °C (Scheme 2). However, if CuI was omitted then the starting material was unchanged even after 5 d. This experiment showed that the reaction could only occur with these substrates when catalyzed by copper.



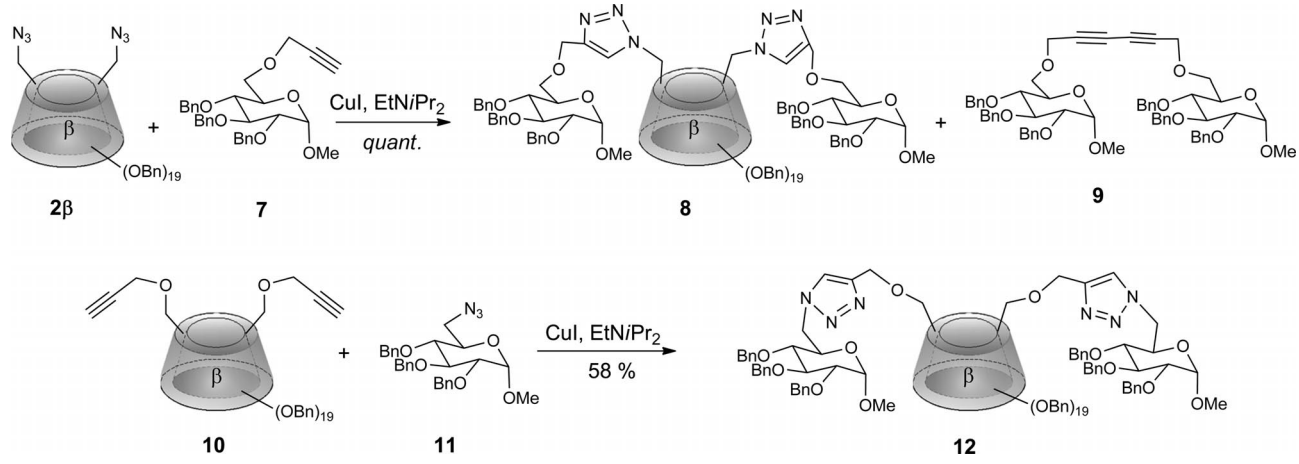
Scheme 1. Preparation of diazide and propargyl ether building blocks. The cyclodextrins were converted into diiodides **1α** or **1β**,[15] or monools **3α** and **3β**[14] by known methods and transformed into **2α**–**β** and **4α**–**β** (Bn = benzyl).



Scheme 2. Reaction of **2β** with simple phenylacetylene.



Scheme 3. Cu-catalyzed Huisgen reaction with bulky acetylene (TIPS = triisopropylsilyl).



Scheme 4. Cu-catalyzed Huisgen reaction with α-D-glucopyranoside derivatives.

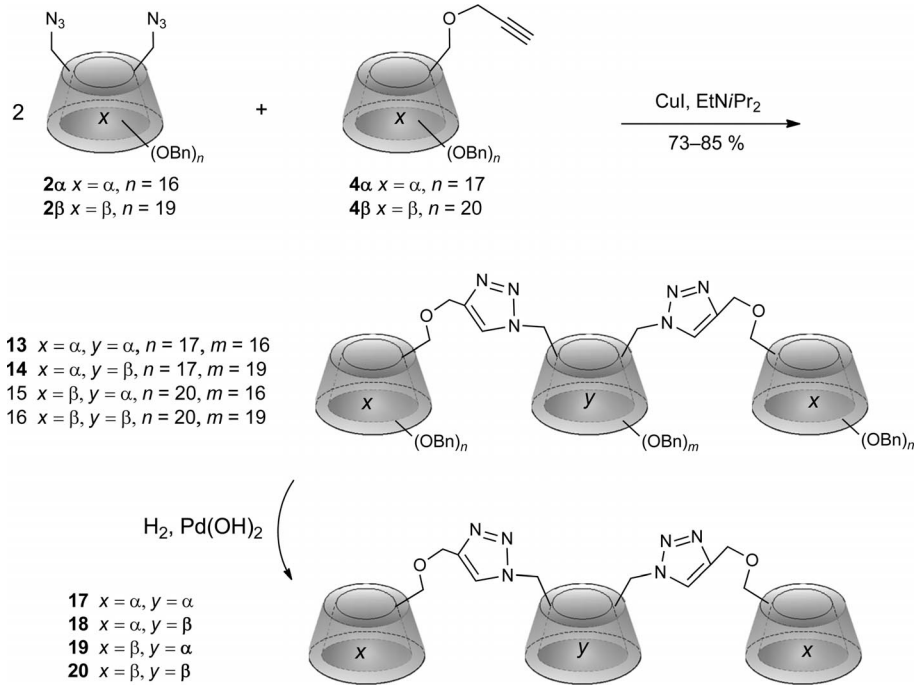
Next, the coupling of diazide **2β** with more bulky substituted alkynes (Scheme 3) was attempted. Reaction of **2β** with triisopropylsilyl acetylene under the same conditions afforded only one compound, **6**, in 58% yield. The presence of a secondary compound was observed during chromatography, which proved to be the Glaser coupling product of terminal alkynes. Additional debenzylation of compound **6** certified the presence of only one isomer.

As an even better model of the intended reaction, azide **2β** was treated with 6-*O*-propargyl ether **7** in the presence of CuI and DIPEA in MeCN/EtOAc (Scheme 4). The desired product **8** was isolated in quantitative yield. The Glaser-coupled byproduct **9** (alkyne–alkyne coupling) was observed as a side product due to the use of a larger excess of alkyne and the presence of oxygen during the reaction.

The alternative click coupling reaction was also tested with building blocks **10** and **11**. Dipropargyl ether cyclodextrin (**10**) was prepared by treatment of β-cyclodextrin diol with *t*BuOK in dimethylformamide at –78 °C and dropwise addition of propargyl bromide. The reaction mixture was allowed to reach room temperature and the progress of the reaction was monitored by TLC until the starting material disappeared. The product was isolated in 64% yield and was used in the copper-catalyzed Huisgen reaction with azide **11** (Scheme 4). This reaction also worked well to give a single triazole product **12** in 58% yield.

Synthesis of the Trimeric Cyclodextrins

Having established a working click chemistry procedure, we proceeded to assemble building blocks **2** and **4** into trimers (Scheme 5). The preparation of the cyclodextrin trimers proceeded smoothly in ethyl acetate/acetonitrile (1:2) with copper(I) iodide and Hünig's base by using 2.1 equivalents of **4α** or **4β** per equivalent of diazide **2α** or **2β** (Scheme 2). Removal of the inorganic copper(I) species was achieved by washing the organic phase with an aqueous solution of ammonium chloride, which eased purification to a great extent. Since the reaction is copper(I) catalyzed, and it is clear from the above model experiment that it can-



Scheme 5. Synthesis of cyclodextrin trimers by click chemistry. From the combination of **4α** or **4β** (2 equiv.) and diazide **2α** and **2β** (1 equiv.) the four possible combination of ditriazoles were prepared.

not proceed otherwise, the structure of the trimeric cyclodextrins must have the 1,4-regioisomeric configuration, as shown in Scheme 2. The four possible combinations of trimeric cyclodextrins, **13**–**16**, were obtained in 73–85% yield after flash chromatography. The click reaction strategy could be performed even on a gram scale without loss of efficiency (e.g., preparation of 1.60 g of **14** occurred in 81% yield). Nevertheless, the yield can be regarded as comparatively low for click reactions, which may be explained by pronounced steric hindrance in the benzylated β-cyclodextrin derivatives.

The remaining step in the synthetic pathway to form trimer cyclodextrins was global deprotection of the benzyl ethers by hydrogenolysis (Scheme 2), which was more difficult than anticipated at first and various strategies were carried out. In Table 1 the screening of reaction conditions for deprotection of trimers **13**–**16** is summarized. At first a procedure that had been applied to deprotection of a benzylated oligosaccharide click product by Gin's group,[23] utilizing transfer hydrogenolysis with ammonium formate as the hydrogen donor and palladium on charcoal, was attempted (Table 1, entry 1), but after two days all benzyl ethers remained intact on the cyclodextrins. More conventional hydrogenolysis under a hydrogen atmosphere was likewise unsuccessful (Table 1, entry 2). With trifluoroacetic acid (TFA) present and a hydrogen pressure of 10–20 bar, hydrogenolysis proceeded at a slow pace (Table 1, entries 3–6); nevertheless, yields were low and one compound, **13**, could not be deprotected. The causes of these problems may be associated with catalyst poisoning from possible impurities and/or the difficulties of finding a solvent mixture that dissolved both starting material and product, avoiding precipitation on the catalyst. There was also the dilemma that acid favored the reaction, but could decompose the glycosidic bonds. Possible traces of sulfur-based catalyst poisons were eliminated by desulfurization with Raney nickel prior to reaction, which had a positive but by no means dramatic effect (Table 1, entries 5–6). The solution was found when Pearlman's catalyst—palladium hydroxide on charcoal—was applied (Table 1, entries 7–10), with 2-methoxyethanol as the solvent. The solvent was perfectly suited for dissolving all stages of the debenzylation process; a task that was insufficiently achieved by the three-phase solvent system of ethyl acetate, methanol, and water. The best results arose when the progress of the deprotection reaction was carefully monitoring, and in some cases extra palladium catalyst was added.

Upon successful deprotection, compounds **10** and **12** (and not **9** and **11**) gave rise to an apparent isomerism that was visible by NMR spectroscopy as doubling of some of the signals and two peaks were also obtained by HPLC. We believe that this is conformational isomerism of the same type as the β-cyclodextrin inversion phenomena recently described by the Monflier group.[24] They observed that a single 6-triazole-substituted glucose residue in the β-cyclodextrin ring could rotate 360°, thereby embedding the triazole deeper in the cavity. Arguments that this is a similar type of isomerization as that in the Monflier case are that doubling of the signals is only observed when the triazoles are substituted on a central β-cyclodextrin and not on α-cyclodextrin. α-Cyclodextrin is presumably to small to undergo such inversion. Second, the ratio of conformational isomers appeared to vary, depending on preparation conditions and solvents. In one preparation of **12**, when comparatively ra-

Table 1. Hydrogenation experiments with different catalysts.

Entry	Starting material	Pd cat.	Raney Ni	Additive	Solvents	Time [d]	H_2 pressure [bar]	Yield [%]
1	**13**	Pd/C	–	NH_4HCO_3	EtOAc/EtOH/H_2O	2	0	–
2	**13**	Pd/C	–	–	EtOAc/EtOH	2	1	–
3	**15**	Pd/C	–	TFA	EtOAc/EtOH/H_2O	2	10	81
4	**14**	Pd/C	–	TFA	EtOAc/EtOH/H_2O	3	15	–
5	**14**	Pd/C	+	TFA	EtOAc/EtOH/H_2O	2	20	38
6	**13**	Pd/C	+	TFA	EtOAc/EtOH/H_2O	2	20	–
7	**13**	$Pd(OH)_2$/C	+	TFA	2-methoxyethanol	16	1	quant.
8	**15**	$Pd(OH)_2$/C	+	TFA	2-methoxyethanol	10	1	91
9	**14**	$Pd(OH)_2$/C	+	TFA	2-methoxyethanol	8	1	quant.
10	**16**	$Pd(OH)_2$/C	+	TFA	2-methoxyethanol	3	1	quant.

pid debenzylation was achieved, only one set of signals were initially observed; however, after some manipulation of the compound another set of signals started to emerge.

Peptide Synthesis

Six peptides were synthesized for this study with the structures FGGGFGGGF, FGGGYGGGF, FGGGNalGGGF, FGGGWGGGF, FGGFGGF, and GGGF, in which F, G, Y, and W are phenylalanine, glycine, tyrosine, and tryptophan, respectively, and Nal is (*S*)-2-(2-naphthylmethyl)glycine. The synthesis was conducted by automated microwave-assisted solid-phase peptide synthesis and subsequent purification was achieved by conventional reverse-phase HPLC. The peptides were synthesized on Rink amide resin by applying a 9-fluorenylmethyl (Fmoc) protecting group strategy and *O*-(benzotriazol-1-yl)tetramethyluronium hexafluorophosphate (HBTU) activation for coupling. Cleavage was achieved by treating the resin with TFA, with triisopropylsilane (TIS) and water as scavengers (95:2.5:2.5 v/v). The yields ranged from 40 to 75%.

Binding Affinity Determination

Initially it was anticipated that isothermal titration calorimetry (ITC) could be used for experimental determination of binding affinities between nonapeptides and cyclodextrins, but it could not accomplished due to difficulties with the solubility of the peptide samples. Therefore, an alternative methodology using surface plasmon resonance was used.[25] The surface plasmon resonance (SPR) technology follows the real-time formation and dissociation of bimolecular complexes on a sensor surface. One of the two components in the binding study under consideration is immobilized on the sensor surface, while the other interacts with it from solution in constant flow over the surface. The binding of the soluble component to the sensor by noncovalent interactions can then be monitored and binding constants determined. To circumvent poor solubility of the peptides, they were immobilized on the sensor.

There are several methods for covalent attachment of a ligand to the surface: the most commonly utilized strategies involve amine coupling, thiol coupling, and aldehyde coupling to attach a ligand to the carboxymethylated dextran matrix covering the gold layer. Based on the fact that all peptides contain a free amino group at the N-terminus, the amine coupling protocol was employed with respect to an existing study. Amine coupling consisted of four successive steps, in which the attachment of all six peptides was achieved after activation of the carboxylic acids of the carboxymethylated dextran matrix of the BIAcore CM5® chip.[25]

With the six chips in hand, titrations were carried out with trimers **17**–**20** and α- and β-cyclodextrin for comparison. In each of the 24 cases, a binding curve was obtained from adding increasing concentrations of the cyclodextrin ligand to the immobilized peptide and recording the maximum response at equilibrium. From these results, apparent association constants were determined and they are shown in Table 2. The results give rise to several observations: (1) α-CD binds with a very different affinity to the different peptides, and does not, as one might anticipate, have a similar K_A value to all compounds that reflects the typical binding for a cyclodextrin to phenyl group. (2) β-CD follows the same trend as α-CD, but binds 1.2 to 1.4 times more tightly with a single exception. (3) With a single exception (compound **17**), all compounds bind GGGF with similar affinity. (4) Trimers **18** and **20** bind all hepta- and nonapeptides more strongly than they bind GGGF; trimer **17**

Table 2. K_A values (in M^{-1}) determined by SPR.

Cyclodextrin	GGGF	FGGFGGF	*FGGGFGGGF*	*FGGGYGGGF*	*FGGGNalGGGF*	*FGGGWGGGF*
α-CD	1060	186	441	960	13	524
β-CD	1320	288	593	1010	162	751
17 (α-α-α)	3280	3790	1210	4690	6100	5480
18 (α-β-α)	1150	5940	6210	5700	5900	5790
19 (β-α-β)	720	1030	1770	1000	1170	834
20 (β-β-β)	1510	4660	3430	5920	4290	5990

behaves very similarly except that it binds GGGF unusually strongly and one of the nonapeptides poorly. (5) Trimer **19** binds all peptides with the essentially the same affinity.

To explain these observations, which may appear confusing, it is necessary to reflect on the properties and possible binding modes of the chip-bound peptides. First, binding with the cyclodextrin trimers may involve one, two, and/or three cyclodextrins, as shown in Figure 2. The actual binding observed may therefore contain contributions from all three binding modes. These different binding modes cannot be discerned by SPR. Second, the larger peptides are very poorly soluble in water, so they are presumably also poorly solvated in chip-attached mode and may coil up upon themselves. It is possible that a strong ligand can uncoil the peptide. By this "coiling" hypothesis, we can explain observations (1) and (2). The high affinity of α- and β-CD for GGGF ($K_A \approx 10^3$ M^{-1}) shows that this peptide has a single exposed phenyl group. (For comparison,[26] the K_A values of phenyl alanine and phenyl alanine amide are 10^1–10^2 M^{-1}). The hepta- and nonapeptides are "coiled" and therefore do not have as readily accessible a phenyl group.

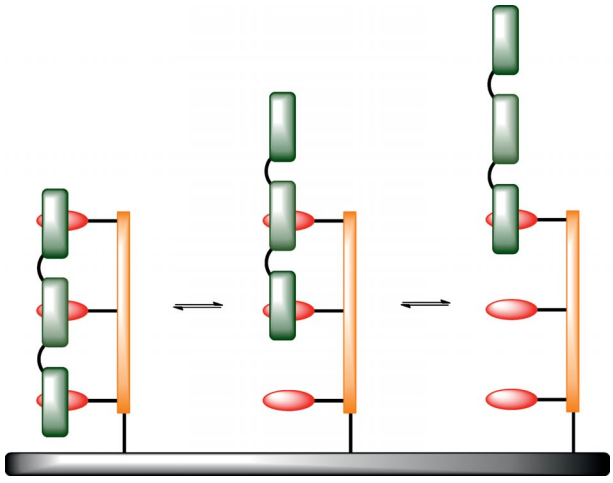


Figure 2. Different possible binding modes of cyclodextrin trimers **17**–**20** (green) with surface-attached peptides (backbone yellow, aromatic side chains red) in the BIAcore. The measured binding constant may be based on contributions for all three binding modes.

This is also why all cyclodextrin trimers bind GGGF with essentially the same affinity (observation 3). The phenyl group is exposed and the cyclodextrin trimer can only bind with one cyclodextrin, which means that binding is similar to that of a simple cyclodextrin.

The higher binding of trimers **18** and **20**, and to some extent **17** (observation 4), to hepta- and nonapeptides ($K_A \approx 6 \times 10^3$ M^{-1}) shows that these compounds can uncoil the peptide and bind to two or more aryl groups (Figure 2). There appears to be little preference for hepta- versus nonapeptide or between different nonapeptides, showing very poor selectivity both for the distance between aryl groups or for the identity. This suggests that this type of binding predominantly involves two cyclodextrin···aryl interactions (Figure 2, middle). Compound **19** is unusual because it has a lower affinity for most of the peptides compared with **17**, **18** and **20**. The binding of **19** is mostly similar to binding by a single cyclodextrin, which suggests that this compound is only able to bind with one cyclodextrin in many cases. Yet **19** must uncoil the larger peptides apparently because all are bound, even if the affinity is lower than for the other trimers (observation 5).

Conclusions

A series of cyclodextrin trimers were successfully prepared by a modified click chemistry methodology for combining benzylated cyclodextrin building blocks. The synthesis of trimers was achieved within 6 synthetic steps and resulted in overall yields of the trimers ranging from 34 (β-β-β) to 52% (α-α-α), starting from unmodified α- or β-cyclodextrin. The copper-catalyzed Huisgen reaction was versatile for all types of alkynes, including small organic alkynes and bulky cyclodextrin–alkyne derivatives.

The cyclodextrin trimers were subsequently investigated for molecular recognition of peptides. The molecular recognition of nonapeptides by cyclodextrin trimers evaluated in this study was unselective, yet better binding affinities were obtained for the cyclodextrin trimers than for the unmodified cyclodextrins. Trimer recognition of nonapeptides was experimentally determined to be superior to the recognition of a heptapeptide. The experimental results, however, suggested poor selectivity of nonapeptides by the cyclodextrin trimers and the recognition of nonapeptides was considered to be unselective. The experimental procedure involved immobilization of peptides, which could have contributed to incomplete overlap of the peptides due to steric hindrance at the surface.

Experimental Section

General: All reactions were carried out in flame-dried glassware under a nitrogen atmosphere unless otherwise stated. Solvents were distilled and/or dried according to standard procedures and the reactions were monitored either by TLC analysis or by MALDI-TOF MS. Flash chromatography was carried out with Merck silica gel 60 (230–400 mesh) as the stationary phase. TLC (Merck silica gel 60, F_{254}) was visualized by UV light or the use of a Ce-Mol solution [cerium(IV) sulfate (10 g) and ammoniummolybdate (15 g) dissolved in 10% aqueous sulfuric acid (1000 mL)] with subsequent heating. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The chemical shifts are reported in ppm downfield to TMS ($\delta = 0$ ppm) for ^{1}H NMR spectroscopy and relative to the solvent peak for ^{13}C NMR spectroscopy. Mass spectra were obtained by using a Micromass LC-TOF mass spectrometer and MALDI-TOF spectra were recorded on a Bruker Daltonics mass spectrometer using a 2,5-dihydroxybenzoic acid (DHBA)-based matrix. Reverse-phase (RP) HPLC was performed on an Agilent 1100 series instrument using a Vydac preparative RP column (C8, 208TP-510), unless otherwise stated.

Hexadeca-*O*-benzyl-6^A,6^D-dideoxy-6^A,6^D-diiodo-α-cyclodextrin (1α): $2^{A–F},3^{A–F},6^{B,C,E,F}$-Hexadeca-*O*-benzyl-α-cyclodextrin[27] (2.00 g, 0.82 mmol), PPh_3 (1.35 g, 5.14 mmol), and imidazole (0.69) were

added to freshly distilled toluene (75 mL) and heated to 75 °C. Then I_2 (1.30 g, 5.14 mmol) was added and the resulting mixture was left stirring overnight at 75 °C under N_2. An equal volume of a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture and it was stirred for 5 min. The organic layer was separated, washed with a saturated aqueous solution of $Na_2S_2O_3$, diluted with EtOAc (150 mL), and washed with water. The organic layer was dried with Na_2SO_4, filtered, concentrated in vacuo, and the resulting oil was purified by flash column chromatography (EtOAc/pentane; 1:5 → 1:3), resulting in a white foam (1.69 g, 0.64 mmol, 78%) ; m.p. 59.1–66.8 °C. ^{1}H NMR ($CDCl_3$): δ = 7.27–7.22 (m, 40 H, Ph), 7.15–7.11 (m, 40 H, Ph), 5.20 (d, J = 10.8 Hz, 2 H), 5.15 (d, J = 14.4 Hz, 2 H), 5.03 (d, J = 11.2 Hz, 2 H), 4.98 (d, J = 3.2 Hz, 2 H), 4.92 (d, J = 3.2 Hz, 2 H), 4.86 (d, J = 10.8 Hz, 2 H), 4.80 (dd, J = 11.2, 10.8 Hz, 4 H), 4.52 (d, J = 3.6 Hz, 2 H), 4.49 (d, J = 3.2 Hz, 2 H), 4.42–4.39 (m, 8 H), 4.37 (d, J = 4.4 Hz, 2 H), 4.13–4.06 (m, 6 H), 3.97 (d, J = 4.8 Hz, 4 H), 3.89 (d, J = 7.2 Hz, 4 H), 3.73 (d, J = 10.0 Hz, 2 H), 3.68–3.64 (m, 2 H), 3.58 (d, J = 10.8 Hz, 2 H), 3.54 (d, J = 8.8 Hz, 2 H), 3.50–3.48 (m, 4 H), 3.46 (t, J = 3.6 Hz, 2 H), 3.43 (d, J = 3.6 Hz, 2 H), 3.36 (dd, J = 3.2, 10.0 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.5, 139.4, 139.3, 138.5, 138.3, 138.2, 138.19, 138.1, 128.5, 128.4, 128.3, 128.28, 128.26, 128.23, 128.1, 127.96, 127.9, 127.88, 127.82, 127.7, 127.6, 127.5, 127.3, 127.1, 127.0, 99.5, 98.5, 84.5, 81.0, 80.8, 80.7, 80.3, 80.2, 79.4, 78.9, 78.7, 75.9, 75.6, 75.4, 73.7, 73.6, 73.0, 72.9, 72.7, 72.0, 71.4, 70.4, 69.6, 69.3 ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{148}H_{154}I_2O_{28}$ $[M + Na]^+$ 2655.8613; found 2654.6694; calcd. for $[M + K]^+$ 2671.8353; found 2670.6228. R_f 0.77 (EtOAc/pentane; 1:3.5).

6^A,6^D-Diazido-hexadeca-*O*-benzyl-6^A,6^D-dideoxy-α-cyclodextrin (2α):[12] Compound **1α** (1.00 g, 0.38 mmol) was stirred with NaN_3 (0.124 g, 1.90 mmol) in DMF (20 mL) at 75 °C overnight under N_2. Then a saturated aqueous solution of NaCl (100 mL) and EtOAc (150 mL) was added to the solution and the resulting mixture was stirred for 5 min. The organic layer was separated and the water phase was extracted with EtOAc. The combined organic phases were washed with water, dried with Na_2SO_4, filtered, and concentrated in vacuo to give an oil, which subsequently was purified by flash column chromatography (EtOAc/pentane; 1:4), resulting in **2α** as a white foam (0.84 g, 0.34 mmol, 89%); m.p. 60.5–64.9 °C. ^{1}H NMR ($CDCl_3$): δ = 7.27–7.22 (m, 40 H, Ph), 7.17–7.11 (m, 40 H, Ph), 5.24 (d, J = 10.8 Hz, 2 H), 5.16 (d, J = 3.2 Hz, 2 H), 5.12 (d, J = 10.8 Hz, 2 H), 4.98 (d, J = 11.2 Hz, 2 H), 4.93 (dd, J = 2.8, 3.2 Hz, 4 H), 4.85 (d, J = 10.8 Hz, 2 H), 4.82 (d, J = 7.2 Hz, 2 H), 4.79 (d, J = 7.2 Hz, 2 H), 4.58 (d, J = 12.4 Hz, 2 H), 4.49 (d, J = 12.0 Hz, 2 H), 4.44 (d, J = 3.2 Hz, 2 H), 4.40 (d, J = 15.6 Hz, 6 H), 4.36 (d, J = 12.0 Hz, 2 H) 4.12 (d, J = 8.4 Hz, 2 H), 4.09 (d, J = 11.6 Hz, 2 H), 4.07 (d, J = 8.0 Hz, 2 H), 4.01 (d, J = 6.8 Hz, 2 H), 3.99 (d, J = 10.4 Hz, 2 H), 3.95 (d, J = 8.8 Hz, 6 H), 3.88 (d, J = 9.6 Hz, 2 H), 3.82 (dd, J = 2.8, 9.6 Hz, 2 H), 3.68 (dd, J = 8.4, 9.6 Hz, 4 H), 3.58 (d, J = 10.4 Hz, 2 H), 3.48 (d, J = 3.6 Hz, 2 H), 3.44 (dd, J = 3.2, 10.0 Hz, 2 H), 3.38 (dd, J = 3.2, 9.6 Hz, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.41, 139.39, 139.36, 138.6, 138.4, 138.3, 138.18, 138.15, 128.49, 128.48, 128.43, 128.36, 128.3, 128.2, 128.1, 128.0, 127.83, 127.77, 127.69, 127.67, 127.65, 127.6, 127.52, 127.48, 127.15, 127.11, 127.05, 126.98, 99.1, 98.9, 98.4, 80.97, 80.92, 80.8, 80.5, 80.2, 79.7, 79.4, 79.2, 78.5, 75.98, 75.85, 75.2, 73.6, 73.56, 73.2, 73.0, 72.7, 72.0, 71.8, 70.9, 69.6, 69.0, 52.4 ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{148}H_{154}N_6O_{28}$ $[M + Na]^+$ 2486.0709; found 2486.7663; calcd. for $[M + K]^+$ 2502.0448; found 2502.9878. R_f 0.77 (EtOAc/pentane; 1:3.5).

Heptadeca-*O*-benzyl-6-*O*-propargyl-α-cyclodextrin (4α): NaH (38 mg, 0.96 mmol) in a solid portion was added to a solution of 2$^{A–F}$,3$^{A–F}$,6$^{B–F}$-heptadeca-*O*-benzyl-α-cyclodextrin (1.00 g, 0.40 mmol) in DMF (10 mL) at 0 °C and stirred for 15 min. Propargyl bromide (0.16 mL, 0.88 mmol) was added dropwise, the reaction mixture was allowed to reach room temperature, and the progress of the reaction was monitored by TLC. The reaction was quenched by adding MeOH (5 mL), diluted with water, and extracted with Et_2O. The combined organic phases were washed with brine, dried with Na_2SO_4, and concentrated in vacuo to give an oil, which was purified by flash column chromatography (EtOAc/pentane; 1:4 → 1:3), resulting in a white foam (0.94 g, 0.37 mmol, 93%); m.p. 54.5–62.9 °C. ^{1}H NMR ($CDCl_3$): δ = 7.27–7.14 (m, 85 H, Ph), 5.20 (dt, J = 3.2, 11.2 Hz, 6 H), 5.14–5.11 (m, 4 H), 5.09 (d, J = 3.2 Hz, 1 H), 5.06 (d, J = 3.2 Hz, 1 H), 4.89 (d, J = 11.2 Hz, 6 H), 4.54 (t, J = 12.8 Hz, 2 H), 4.51–4.44 (m, 15 H), 4.43 (d, J = 4.0 Hz, 2 H), 4.39 (dd, J = 1.6, 2.8 Hz, 2 H), 4.36 (dd, J = 2.4, 3.2 Hz, 1 H), 4.19 (d, J = 1.6 Hz, 1 H), 4.17–4.12 (m, 6 H), 4.10 (d, J = 4.0 Hz, 1 H), 4.07 (d, J = 3.6 Hz, 2 H), 4.06–4.02 (m, 7 H), 4.00 (d, J = 2.4 Hz, 2 H), 3.96–3.94 (m, 8 H), 3.65 (dd, J = 10.4, 11.6 Hz, 2 H), 3.57 (d, J = 10.8 Hz, 2 H), 3.55 (d, J = 7.6 Hz, 1 H), 3.52 (dd, J = 3.2, 6.4 Hz, 4 H), 3.50 (d, J = 3.2 Hz, 2 H), 3.46 (dd, J = 3.2, 9.6 Hz, 2 H), 2.23 (t, J = 2.4 Hz, 1 H, C≡C-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.3, 138.3, 138.2, 138.19, 138.15, 138.12, 128.4, 128.39, 128.3, 128.2, 128.1, 127.9, 127.8, 127.74, 127.70, 127.66, 127.61, 127.59, 127.54, 127.3, 127.2, 127.1, 126.9, 98.7, 98.62, 98.59, 98.53, 80.9, 80.8, 79.8, 79.4, 79.3, 79.2, 79.1, 79.06, 79.00, 78.9, 75.6, 75.5, 75.4, 74.7, 73.4, 73.3, 72.8, 72.7, 72.6, 71.5, 71.4, 69.1, 69.0, 58.5 ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{158}H_{164}O_{30}$ $[M + Na]^+$ 2564.1205; found 2564.0170; calcd. for $[M + K]^+$ 2580.0995; found 2580.3392. R_f 0.66 (EtOAc/pentane; 1:3.5).

6^A,6^D-Diazido-nonadeca-*O*-benzyl-6^A,6^D-dideoxy-β-cyclodextrin (2β): Compound **1β** (2.00 g, 0.65 mmol) was stirred with NaN_3 (0.21 g, 3.26 mmol) in DMF (40 mL) at 75 °C overnight under N_2. A saturated aqueous solution of NaCl (200 mL) and EtOAc (250 mL) was added to the solution and the resulting mixture was stirred for 5 min. The organic layer was separated and the water phase was extracted with EtOAc. The combined organic phases were washed with water, dried with Na_2SO_4, filtered, and concentrated in vacuo to give an oil, which subsequently was purified by flash column chromatography (EtOAc/pentane; 1:4), resulting in a white foam (1.67 g, 0.58 mmol, 89%); m.p. 54.3–60.5 °C. ^{1}H NMR ($CDCl_3$): δ = 7.25–7.10 (m, 95 H), 5.27 (d, J = 4.0 Hz, 1 H), 5.16 (d, J = 3.6 Hz, 1 H), 5.13 (d, J = 3.6 Hz, 2 H), 5.10 (t, J = 4.0 Hz, 2 H), 5.07–5.05 (m, 2 H), 5.04 (d, J = 3.6 Hz, 1 H), 4.99 (d, J = 3.6 Hz, 1 H), 4.96 (d, J = 11.2 Hz, 1 H), 4.82 (d, J = 11.2 Hz, 1 H), 4.77 (d, J = 11.6 Hz, 1 H), 4.73–4.69 (m, 7 H), 4.57 (dd, J = 9.6, 11.6 Hz, 2 H), 4.50–4.38 (m, 19 H), 4.10–3.88 (m, 25 H), 3.67 (d, J = 8.4 Hz, 2 H), 3.62 (d, J = 10.4 Hz, 2 H), 3.59 (d, J = 10.4 Hz, 4 H), 3.54 (dd, J = 2.8, 10.4 Hz, 2 H), 3.50 (dd, J = 3.6, 8.8 Hz, 2 H), 3.47–3.43 (m, 5 H), 3.39 (dt, J = 3.2, 8.8 Hz, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.43, 139.35, 139.22, 139.18, 139.15, 138.6, 138.5, 138.38, 138.35, 138.29, 138.25, 138.2, 138.1, 128.7, 128.64, 128.60, 128.48, 128.46, 128.4, 128.34, 128.32, 128.29, 128.18, 128.16, 128.09, 128.05, 128.01, 127.95, 127.9, 127.8, 127.7, 127.62, 127.59, 127.55, 127.3, 127.2, 127.12, 127.08, 127.0, 98.9, 98.74, 98.68, 98.66, 98.4, 98.2, 98.0, 81.0, 80.90, 80.85, 80.4, 80.0, 79.8, 79.5, 79.41, 79.39, 79.36, 79.0, 78.90, 78.88, 78.8, 78.2, 77.8, 76.0, 75.9, 75.8, 75.3, 75.08, 75.05, 73.5, 73.4, 73.02, 72.99, 72.97, 72.8, 72.7, 71.9, 71.8, 71.7, 71.6, 70.92, 70.86, 69.62, 69.57, 69.4, 69.10, 69.06, 52.4 ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{175}H_{182}N_6O_{33}$ $[M + Na]^+$ 2918.2646; found 2918.4578; calcd. for $[M + K]^+$ 2934.2385, found 2934.7091. R_f 0.66 (EtOAc/pentane; 1:3).

Icosa-*O*-benzyl-6-*O*-propargyl-β-cyclodextrin (4β): NaH (0.016 g, 0.41 mmol) in a solid portion was added to a solution of **3β** (0.50 g, 0.17 mmol) in DMF (4.0 mL) at 0 °C and stirred for 15 min. Propargyl bromide (0.02 mL, 0.19 mmol) was added dropwise, the reaction mixture was allowed to reach room temperature, and the progress of the reaction was monitored by TLC. The reaction was quenched by adding MeOH (5 mL), diluted with water, extracted with Et_2O, the combined organic phases were washed with brine, dried with Na_2SO_4, concentrated in vacuo to give an oil, which was purified by flash column chromatography (EtOAc/pentane; 1:4 → 1:3) resulting in a white foam (0.44 g, 0.15 mmol, 88%); m.p. 55.1–62.2 °C. ^{1}H NMR ($CDCl_3$): δ = 7.24–7.15 (m, 10 0 H, Ph), 5.25 (d, J = 3.6 Hz, 1 H), 5.21 (d, J = 3.6 Hz, 1 H), 5.17 (d, J = 3.6 Hz, 1 H), 5.15 (d, J = 3.6 Hz, 1 H), 5.14 (d, J = 4.0 Hz, 1 H), 5.11 (t, J = 3.6 Hz, 3 H), 5.07 (d, J = 10.8 Hz, 4 H), 5.03 (d, J = 8.4 Hz, 1 H), 4.98 (d, J = 6.0 Hz, 1 H), 4.76 (d, J = 11.2 Hz, 7 H), 4.55 (d, J = 12.4 Hz, 1 H), 4.49–4.46 (m, 12 H), 4.43 (d, J = 6.4 Hz, 5 H), 4.41 (d, J = 2.4 Hz, 2 H), 4.38 (d, J = 8.0 Hz, 7 H), 4.05–3.93 (m, 32 H), 3.86 (d, J = 8.4 Hz, 1 H), 3.81 (d, J = 8.8 Hz, 1 H), 3.68 (d, J = 10.0 Hz, 1 H), 3.62 (d, J = 10.4 Hz, 1 H), 3.55 (d, J = 10.4 Hz, 5 H), 3.50 (d, J = 9.2 Hz, 2 H), 3.48 (d, J = 4.0 Hz, 3 H), 3.45 (dd, J = 2.0, 3.6 Hz, 3 H), 3.42 (d, J = 3.6 Hz, 1 H), 2.19 (t, J = 2.0 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.53, 139.50, 138.61, 138.55, 138.46, 129.10, 129.04, 128.99, 128.97, 128.94, 128.90, 128.79, 128.72, 128.53, 128.38, 128.205, 128.13, 128.09, 128.05, 127.99, 127.80, 127.77, 127.66, 127.56, 127.15, 98.87, 98.82, 98.67, 98.62, 98.56, 98.52, 81.23, 81.12, 81.12, 81.06, 81.00, 79.94, 79.51, 79.30, 79.19, 79.14, 79.02, 78.98, 78.89, 78.82, 78.13, 75.77, 75.68, 75.65, 75.48, 75.39, 75.03, 73.49, 72.98, 72.91, 72.87, 72.77, 71.80, 71.73, 71.59, 71.23, 71.21, 69.50, 69.42, 69.33, 69.09, 69.07, 58.64 ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{185}H_{192}O_{35}$ $[M + Na]^+$ 2996.3142; found 2995.9890; calcd. for $[M + K]^+$ 3012.2881; found 3012.2943. R_f 0.68 (EtOAc/pentane; 1:3).

$2^{A–F}$,$3^{A–F}$,$6^{B,C,E,F}$-Hexadecakis-*O*-benzyl-$6^{A,D}$-di-*C*-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-β-cyclodextrin (5): Derivative **2β** (150 mg, 0.052 mmol) was dissolved in MeCN (5 mL) and EtOAc (2.5 mL), together with CuI (2 equiv.), DIPEA (3 equiv.), and phenylacetylene (21.2 mg, 0.207 mmol, 4 equiv.) were added and the resulting mixture was stirred overnight at 40 °C. The reaction mixture was poured into a saturated aqueous solution of NH_4Cl (10 mL) and water (20 mL). The organic phase was separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine, dried with MgSO4, filtered, and the solvents were evaporated. The resulting oil was purified by flash chromatography (EtOAc/pentane; 1:4 → 1:2), which gave **5** as a clear solid residue (129.4 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.49 (s, 2 H, triazole H), 7.25–6.93 (m, 105 H, Ph), 5.83 (d, J = 3.2 Hz, 1 H), 5.55 (d, J = 3.15 Hz, 1 H), 5.32–5.12 (m, J = 11.2 Hz, 5 H), 5.08–4.92 (m, 5 H), 4.88–4.64 (m, 7 H, CHPh), 4.50–4.32 (m, 26 H, CHPh), 4.24–3.82 (m, 28 H, 7 × 3-H, 7 × 4-H, 7 × 5-H, 7 × 6-H), 3.70–3.25 (m, 12 H, 7 × 6-H, 5 × 2-H), 2.93–2.89 (d, 2 H, 2 × 2-H) ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{191}H_{194}N_6O_{33}$ $[M + Na]^+$ 2690.1648; found 2690.0348; calcd. for $[M + K]^+$ 2706.1387; found 2706.2119.

Nonadecakis-*O*-benzyl-6^A,6^D-di-*C*-[4-(triisopropylsilyl)-1*H*-1,2,3-triazol-1-yl]-β-cyclodextrin (6): Following the same procedure as that used for **5**, derivative **2β** (600 mg, 0.208 mmol) was coupled with triisopropylsilyl acetylene (151.2 mg, 0.829 mmol) to obtain **6** as a clear solid residue (391 mg, 58%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.48, 7.46 (s, 2 H, triazol H), 7.24–6.93 (m, 105 H, Ph), 5.73 (d, J = 3.4 Hz, 1 H, 1-H), 5.52 (d, J = 3.2 Hz, 1 H, 1-H), 5.49 (d, J = 3.5 Hz, 1 H, 1-H), 5.40 (d, J = 3.3 Hz, 1 H, 1-H), 5.21–4.97 (m, 12 H, CHPh, 3 × 1-H), 4.84–4.19 (m, 35 H, CHPh, 6-H), 4.12–3.72 (m, 20 H, 3-H, 4-H, 6-H), 3.58–3.37 (m, 14 H, 5-H, 5 × 2-H), 3.19–3.13 (dd, 2 H, 2 × 2-H), 1.29 (k, 6 H, 6 × SiCHMe), 1.06 (d, 36 H, 12 × CH_3CH) ppm. ^{13}C NMR (75 MHz, CDCl3): δ = 139.6, 139.2, 138.6, 138.5, 138.4, 138.2 ($C_{quat.}$ triazole, C_{ipso}), 128.7, 128.5, 128.4, 128.2, 128.1, 127.8, 127.7 (Ar), 99.6, 98.6, 98.5, 98.3, 98.1, 97.6 (1-C), 80.8, 80.6, 77.5, 73.6, 73.5, 72.8, 72.7, 69.5, 18.9 (CH_3), 11.3 [$C(CH_3)_2$] ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{197}H_{226}N_6O_{33}Si_2$ $[M + 2H]^+$ 3264.108; found 3264.41.

Nonadecakis-*O*-benzyl-6^A,6^D-di-*C*-[4-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucosidyl)-1*H*-1,2,3-triazol-1-yl]-β-cyclodextrin (8): Diazide **2β** (202 mg, 70 μmol) and methyl 2,3,4-tri-*O*-benzyl-6-*O*-propargyl α-D-glucoside (326 mg, 10 equiv., 0.70 mmol) were dissolved in MeCN/EtOAc (2:1, 2 mL). CuI (27 mg, 2 equiv., 140 μmol) and diisopropylethylamine (5 equiv., 60 μL) were added and the reaction was mixed in a closed flask for 2 weeks. The yellow crude reaction mixture was diluted with EtOAc, washed with a saturated aqueous solution of NH_4Cl, HCl (1 M), a saturated aqueous solution of $NaHCO_3$, and brine. After concentration in vacuo, the crude product was purified by flash chromatography (EtOAc/pentane 1:5 to 1:2) to give the desired product in quantitative yield. The Glaser coupled byproduct (alkyne–alkyne coupling) was isolated as a side product. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.51–6.98 (m, 127 H, Ar, triazole-H), 5.61 (d, J = 3.2 Hz, 1 H, 1-H), 5.53 (d, J = 3.2 Hz, 1 H, 1-H), 5.36–5.30 (m, 3 H, CHPh, 1-H), 5.25 (d, J = 3.0 Hz, 1 H, 1-H), 5.19 (d, J = 3.0 Hz, 1 H, 1-H), 5.17–5.10 (m, 4 H), 5.07 (d, J = 10.3 Hz, 1 H, CHPh), 5.02–4.98 (m, 3 H, 7 H, CHPh, 6-H), 4.88 (t, J = 10.4 Hz, 8 H, 6-H), 4.84–4.76 (m, 8 H, CHPh, 3-H), 4.75–4.71 (m, 4 H, CHPh), 4.67 (d, J = 12.2 Hz, 6 H, CHPh), 4.64–4.35 (m, 42 H, 1-H, 6-H), 4.27–4.13 (m, 11 H, CHPh, 2-H), 4.09–3.87 (m, 24 H, CHPh, 3-H, 6-H), 3.87–3.70 (m, 10 H, 4-H), 3.65 –3.41 (m, 18 H, 5-H, 4-H), 3.37 (s, 6 H), 3.34–3.28 (m, 2 H, 2-H), 3.26 (dd, J = 9.7, 3.4 Hz, 1 H, 2-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 144.1 (C_{quat} triazole), 138.9, 138.30, 138.26, 138.22, 138.17, 138.1 (C_{ipso}, Ar), 128.50, 128.48, 128.46, 128.43, 128.38, 128.33, 128.28, 128.25, 128.20, 128.16, 128.12, 128.09, 128.07, 128.02, 127.98, 127.94, 127.91, 127.87, 127.79, 127.75, 127.71, 127.63, 127.58, 127.52, 127.4, 127.3, 127.2, 127.1, 127.0 (Ar), 125.2 (CH triazole), 99.1, 98.4, 98.2, 97.7 (C-1), 82.1 (C-3), 80.4 (C-4), 79.9 (C-5), 78.9, 77.3, 77.1, 76.9, 75.8, 75.1 (CH_2Ph), 73.6, 73.42, 73.38, 73.1, 72.8, 72.54, 72.47, 72.3, 71.9, 71.5 (C-3, C-2), 70.1 (C-4, C-5), 69.2 (C-6), 65.0 (C-6), 55.3 (CH_3), 50.7 (C-6) ppm. MS (MALDI-TOF): m/z (%) = 3901.82 (100.0) $[M]^+$.

1,6-Di-*O*-(methyl 2,3,4-tri-*O*-benzyl-6-yl-α-D-glucopyranosidyl)-2,4-hexadiyne (9): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.29 (m, 30 H, Ar), 5.02 (d, J = 10.8 Hz, 2 H, Bn) 4.86 (m, 4 H, Bn) 4.84 (d, J = 12.0 Hz, 2 H, Bn), 4.69 (d, J = 12.1 Hz, 1 H, Bn), 4.65 (d, J = 10.8 Hz, 2 H, Bn), 4.63 (d, J = 3.3 Hz, 2 H, 1-H, 1 H′), 4.25 (d, J = 16.6 Hz, 2 H, CH_2-≡), 4.18 (d, J = 16.6 Hz, 2 H, CH_2-≡), 4.01 (br. t, J = 9.3 Hz, 2 H, 3-H, 3′-H), 3.79 (m, 4 H, 6-H, 6′-H, 5-H, 5′-H), 3.63 (m, 4 H, 6-H, 6′-H, 4-H, 4′-H), 3.56 (m, 2 H, 2-H, 2′-H), 3.40 (s, 6 H, Me) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 139.0, 138.4, 138.3 (6 C_{ipso}), 128.6–127.8 (30 C, Ar), 98.5 (2 C, C1, C1′), 82.2 (2 C, C3, C3′), 79.9 (2 C, C2, C2′), 77.4 (2 C, C4, C4′), 75.9 (2 C, Bn), 75.4 (2 C, Bn), 75.3 (2 C, Bn), 73.6 (alkyn), 71.0 (alkyn), 69.9 (2 C, C5, C5′), 68.5 (2 C, C6, C6′), 59.2 (2 C, 2 × CH_2-≡), 55.4 (2 C, 2 × Me) ppm.

$2^{A–G}$,$3^{A–G}$,$6^{B,C,E,F,G}$-Nonadecakis-*O*-benzyl-$6^{A,D}$-di-*O*-propargyl-β-cyclodextrin (10): *t*BuOK (0.122 g, 2.63 mmol) and propargyl bromide (0.44 mL, 4 mmol; dropwise) were added to a solution of β-cyclodextrin diol (0.751 g, 0.263 mmol) in DMF (5 mL) at –78 °C. The reaction mixture was allowed to reach room temp. and the progress of the reaction was monitored by TLC. The reaction was

quenched by adding water and was extracted with EtOAc. The combined organic phases were washed with brine, dried with Na_2SO_4, concentrated in vacuo, and purified by flash chromatography (EtOAc/pentane; 1:4 → 1:3), resulting in **10** as a clear solid residue (0.497 g, 0.17 mmol, 64%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33–7.13 (m, 95 H, Ph), 5.34 (d, J = 3.6 Hz, 1 H, 1-H), 5.28 (d, J = 3.2 Hz, 1 H, 1-H), 5.26 (d, J = 3.6 Hz, 1 H, 1-H), 5.20–5.10 (m, 7 H, CHPh), 5.04 (d, J = 11.6 Hz, 2 H, CHPh), 5.0 (d, J = 11.2 Hz, 1 H, CHPh), 4.85–4.80 (m, 7 H), 4.57–4.46 (m, 28 H), 4.09–3.94 (m, 28 H), 3.77–3.68 (m, 7 H), 3.63 (d, J = 10.8 Hz, 2 H), 3.58 (d, J = 3.2 Hz, 2 H), 3.55–3.48 (m, 4 H), 2.30 (t, J = 2.4 Hz, 1 H, CH), 2.28 (t, J = 2.4 Hz, 1 H, CH) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 139.4–138.2 (C_{ipso}, Ar), 128.40, 128.37, 128.23, 128.22, 128.07, 128.04, 127.99, 127.94, 127.89, 127.86, 127.70, 127.66, 127.62, 127.60, 127.57, 127.50, 127.46, 127.41, 127.23, 127.20, 127.10, 127.01 (Ar), 98.6, 98.5, 98.4, 98.3 (C-1), 80.9, 79.77 (C_{quat}), 79.75, 79.1, 78.9, 78.8, 77.4, 77.1 (CH), 76.9, 75.8, 75.7, 75.6, 75.3, 75.1, 75.0, 74.9 (CH_2Ph), 73.3, 73.3, 73.0, 72.91, 72.85, 72.78, 72.75, 72.72, 72.6, 71.5, 71.0, 69.3, 69.2, 68.9, 58.53 (C-6), 58.48 (C-6) ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{181}H_{188}O_{35}$ [M + Na$^+$] 2944.2829; found 2943.9462; calcd. for [M + K$^+$] 2960.2568; found 2960.5916.

6^{A,D}-Di-*O*-{methyl-4-[*N*-(methyl 6-deoxy-2,3,4-tri-*O*-benzyl-α-D-glucopyranosidyl)]triazolyl}-2$^{A–G}$,3$^{A–G}$,6^{B,C,E,F,G}-nonadecakis-*O*-benzyl-β-cyclodextrin (12): Compound **10** (101 mg, 34.6 μmol) and the methyl 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranoside (169 mg, 10 equiv., 0.35 mmol) were dissolved in MeCN/EtOAc (2:1, 4 mL) and CuI (13 mg, 2 equiv., 69 μmol) was added together with diisopropylethylamine (5 equiv., 30 μL). The reaction was left at room temp. for 2 weeks to give a dark solution, which was diluted (EtOAc) and washed with NH_4Cl (satd. solution) and brine, followed by drying of the organic layer, and concentration in vacuo to give a crude product, which was purified by flash chromatography (EtOAc/pentane; 1:4 → 1:2) to give **12** as a clear solid residue (79 mg, 58%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.51–6.98 (m, 127 H, Ar, triazole-H), 5.33 (d, J = 3.4 Hz, 1 H, 1-H), 5.26 (d, J = 3.5 Hz, 2 H, 1-H), 5.24 (d, J = 3.6 Hz, 1 H, 1-H), 5.21 (d, J = 10.4 Hz, 1 H, CHPh), 5.19 (d, J = 10.6 Hz, 1 H, CHPh), 5.17 (d, J = 3.6 Hz, 2 H, 1-H), 5.10–5.00 (m, 8 H, 2× 1-H, CHPh), 4.91 (dd, J = 10.8, 1.1 Hz, 4 H, CHPh), 4.87- 4.66 (m, 14 H, CHPh), 4.66–4.58 (m, 4 H, CHPh), 4.58–4.33 (m, 30 H, 2× 1-H, 6-H), 4.25–3.76 (m, 30 H, 3-H, 5-H), 3.57 (dt, J = 13.7, 6.7 Hz, 6 H), 3.52–3.44 (m, 8 H, 2-H), 3.41 (dd, J = 9.5, 3.4 Hz, 2 H, 2-H), 3.16 (2s, 6 H, CH_3), 3.14–3.10 (m, 3 H, 4-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 144.8 (C_{quat} triazole), 139.32, 139.30, 139.26, 139.24, 139.19, 138.55, 138.51, 138.48, 138.41, 138.34, 138.32, 138.28, 138.25, 138.23, 138.20, 137.98, 137.95, 134.7 (C_{ipso}, Ar), 129.8, 129.0, 128.57, 128.52, 128.47, 128.38, 128.36, 128.34, 128.16, 128.13, 128.02, 127.98, 127.94, 127.81, 127.79, 127.75, 127.66, 127.58, 127.55, 127.49, 127.44, 127.3, 127.2, 127.1 (Ar), 126.96, 126.94, 123.85 (CH triazole), 98.63, 98.58, 98.5, 98.4, 98.3 (7× C-1), 97.93, 97.91 (2× C-1), 81.9, 80.9 (C-3), 79.9, 79.2 (C-2), 78.1, 77.3, 77.1, 76.8 (C-2, C-4), 75.8, 75.0, 73.4, 73.3, 73.2, 72.6 (CH_2Ph,C-6), 71.5 (C-5), 69.3 (CH_2), 69.1 (C-2), 65.0 (C-6), 55.3 (CH_3), 50.5 (C-6-triazole) ppm. MS (MALDI-TOF): m/z (%) = 3924.43 (100.0) [M + Na]$^+$.

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-(2$^{A–F}$,3$^{A–F}$,6$^{B–F}$-heptadeca-*O*-benzyl-α-cyclodextrin-6^A-oxy)-1,2,3-triazolyl-4-methyl]}hexadeca-*O*-benzyl-α-cyclodextrin (13): CuI (0.039 g, 0.20 mmol) and DIPEA (0.05 mL, 0.30 mmol) were added to a solution of **4α** (0.54 g, 0.21 mmol) and **2α** (0.25 g, 0.10 mmol) in MeCN (2 mL) and EtOAc (2 mL) and the resulting mixture was stirred until TLC showed no diazide. The reaction was then quenched by adding water (20 mL) and a saturated aqueous solution of NH_4Cl (10 mL). The aqueous layer was extracted three times with EtOAc, the combined organic phases were washed with brine, dried with $MgSO_4$, and the solvent removed in vacuo. The resulting oil was purified by flash column chromatography (EtOAc/pentane; 1:4 → 1:2) to give **13** as a white foam (0.63 g, 0.08 mmol, 83%); m.p. 81.6–85.9 °C. ^{1}H NMR ($CDCl_3$): δ = 7.30 (s, 2 H, triazole-H), 7.24–7.02 (m, 250 H), 5.76 (d, J = 3.2 Hz, 1 H), 5.55 (d, J = 3.2 Hz, 1 H), 5.37 (d, J = 10.0 Hz, 1 H), 5.27 (dd, J = 2.0, 10.8 Hz, 2 H), 5.02 (t, J = 2.8 Hz, 2 H), 5.16–5.14 (m, 6 H), 5.11 (d, J = 3.2 Hz, 2 H), 5.09–5.04 (m, 6 H), 4.94 (d, J = 2.8 Hz, 1 H), 4.89 (s, 2 H), 4.87 (d, J = 2.0 Hz, 8 H), 4.85–4.79 (m, 18 H), 4.75 (d, J = 10.0 Hz, 2 H), 4.70 (d, J = 12.0 Hz, 2 H), 4.60 (dd, J = 4.8, 12.0 Hz, 2 H), 4.54 (d, J = 6.0 Hz, 2 H), 4.49 (d, J = 2.0 Hz, 6 H), 4.46–4.40 (m, 30 H), 4.38 (d, J = 2.8 Hz, 2 H), 4.36 (d, J = 3.6 Hz, 6 H), 4.32 (dd, J = 3.2, 4.4 Hz, 6 H), 4.29 (d, J = 3.2 Hz, 2 H), 4.20 (d, J = 11.6 Hz, 2 H), 4.15–4.05 (m, 50 H), 3.95 (t, J = 11.6 Hz, 20 H), 3.88 (d, J = 7.6 Hz, 6 H), 3.69 (d, J = 10.8 Hz, 2 H), 3.60–3.56 (m, 6 H), 3.52–3.44 (m, 30 H), 3.20 (dd, J = 3.6, 9.6 Hz, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.6, 139.3, 139.2, 139.0, 138.7, 138.6, 138.52, 138.48, 138.46, 130.0, 129.2, 128.61, 128.56, 128.49, 128.44, 128.38, 128.35, 128.33, 128.29, 128.2, 128.1, 127.91, 127.89, 127.85, 127.5, 127.4, 127.3, 98.7, 81.4, 81.19, 81.16, 81.07, 81.06, 81.02, 76.1, 74.9, 74.7, 74.3, 74.2, 73.9, 73.66, 73.63, 73.59, 73.65, 73.5, 73.2, 72.9, 72.79, 72.74, 72.72, 72.69, 72.64, 72.58, 72.56, 72.51, 71.90, 71.87, 71.85, 71.65, 71.61, 71.51, 71.48, 69.3, 69.2 ppm. HRMS (MALDI-TOF): m/z calcd. for $C_{414}H_{482}N_6O_{88}$ [M + Na]$^+$ 7568.3324; found 7568.6194; calcd. for [M + K]$^+$ 7584.3063; found 7583.9533. R_f 0.29 (EtOAc/pentane; 1:2.5).

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-(2$^{A–F}$,3$^{A–F}$,6$^{B–F}$-heptadeca-*O*-benzyl-α-cyclodextrin-6^A-oxy)-1,2,3-triazolyl-4-methyl]}nonadeca-*O*-benzyl-β-cyclodextrin (14): To a solution of alkyn **4α** (1.35 g, 0.53 mmol) and diazide **2β** (0.73 g, 0.25 mmol) in 4 mL of MeCN and 4 mL of EtOAc, CuI (97 mg, 0.51 mmol) and DIPEA (0.132 mL, 0.76 mmol) was added and the resulting mixture was left stirring until TLC showed no diazide. The reaction was then quenched by adding 20 mL of water and 10 mL of satd. aq. NH_4Cl. The aqueous layer was extracted thrice with EtOAc, the combined organic phases were washed with brine, dried with $MgSO_4$ and the solvent removed in vacuo. The resulting oil was purified by flash column chromatography (EtOAc/pentane; 1:4 → 1:2) giving 1.61 g of **14** (0.20 mmol, 81%) as a white foam. The compound was a 10:9 mixture of isomers. Mp 83.0–87.7 °C. ^{1}H NMR ($CDCl_3$): δ = 7.31 (s, 4 H, triazole-H), 7.42–6.98 (m, 504 H, -Ph), 5.67 (d, J = 3.2 Hz, 1 H), 5.57 (d, J = 2.4 Hz, 1 H), 5.43 (d, J = 2.8 Hz, 0.90 H), 5.27 (dd, J = 6.4, 10.8 Hz, 7 H), 5.22 (d, J = 2.4 Hz, 0.90 H), 5.20–5.18 (m, 11 H), 5.14–5.05 (m, 30 H), 5.02 (d, J = 3.2 Hz, 2 H), 4.92–4.89 (m, 6 H), 4.85 (t, J = 9.2 Hz, 21 H), 4.75 (d, J = 10.0 Hz, 4 H), 4.70 (d, J = 11.2 Hz, 4 H), 4.66 (s, 3 H), 4.61 (dd, J = 1.2, 10.4 Hz, 2 H), 4.57–4.55 (m, 2 H), 4.52 (t, J = 4.4 Hz, 6 H), 4.48–4.44 (m, 40 H), 4.42 (d, J = 5.2 Hz, 25 H), 4.39–4.33 (m, 39 H), 4.28 (d, J = 12.0 Hz, 11 H), 4.22 (d, J = 12.0 Hz, 7 H), 4.15–4.10 (m, 24 H), 4.07 (d, J = 3.6 Hz, 10 H), 4.06–3.98 (m, 60 H), 3.92 (d, J = 10.8 Hz, 27 H), 3.86 (d, J = 10.0 Hz, 16 H), 3.78 (d, J = 7.6 Hz, 8 H), 3.72 (d, J = 11.6 Hz, 7 H), 3.58 (d, J = 10.0 Hz, 7 H), 3.55–3.53 (m, 6 H), 3.49–3.41 (m, 60 H), 3.22 (dd, J = 3.2, 10.0 Hz, 3.6 H), 3.17 (dd, J = 2.8, 10.0 Hz, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.53, 139.47, 138.55, 138.50, 138.47, 138.37, 138.32, 129.11, 129.06, 129.04, 128.91, 128.74, 128.55, 128.41, 128.28, 128.23, 128.06, 127.92, 127.75, 127.67, 127.58, 127.45, 127.37, 127.27, 127.25, 127.15, 127.10, 98.67, 98.60, 98.58, 98.55, 78.90, 73.50, 73.46, 73.34, 73.30, 73.04, 72.73, 72.65, 72.62, 72.57, 72.51, 72.48, 71.78, 71.74, 71.71, 71.65, 71.51, 71.48, 71.45, 71.39,

71.35, 69.12, 69.02 ppm. HRMS (MALDI-TOF): *m*/*z* calcd. for $C_{491}H_{510}N_6O_{93}$ [M + Na]$^+$ 8000.5260; found 8000.1963; calcd. for [M + K]$^+$ 8016.5000; found 8016.0023. R_f 0.26 (EtOAc/pentane; 1:2.5).

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-($2^{A–G}$,$3^{A–G}$,$6^{B–G}$-icosa-*O*-benzyl-β-cyclodextrin-6^A-oxy)-1,2,3-triazolyl-4-methyl]}hexadeca-*O*-benzyl-α-cyclodextrin (15): CuI (19.1 mg, 0.10 mmol) and DIPEA (0.025 mL, 0.15 mmol) were added to a solution of **4β** (0.32 g, 0.11 mmol) and **2α** (0.12 g, 0.05 mmol) in MeCN (2 mL) and EtOAc (2 mL) and the resulting mixture was stirred until TLC showed no diazide. The reaction was then quenched by adding water (20 mL) and a saturated aqueous solution of NH_4Cl (10 mL). The aqueous layer was extracted three times with EtOAc, the combined organic phases were washed with brine, dried with $MgSO_4$, and the solvent removed in vacuo. The resulting oil was purified by flash column chromatography (EtOAc/pentane; 1:4 → 1:2) to give **15** as a white foam (0.36 g, 0.043 mmol, 85%); m.p. 81.7–88.5 °C. ^{1}H NMR ($CDCl_3$): δ = 7.25 (s, 2 H), 7.23–7.03 (m, 280 H, Ph), 5.77 (d, *J* = 2.0 Hz, 2 H), 5.53 (d, *J* = 3.2 Hz, 2 H), 5.37 (d, *J* = 10.0 Hz, 2 H), 5.29 (d, *J* = 3.6 Hz, 4 H), 5.24 (d, *J* = 2.8 Hz, 6 H), 5.21 (t, *J* = 4.0 Hz, 6 H), 5.16 (d, *J* = 3.6 Hz, 2 H), 5.13–5.10 (m, 7 H), 5.07 (d, *J* = 7.6 Hz, 4 H), 4.98 (t, *J* = 10.8 Hz, 10 H), 4.82–4.69 (m, 30 H), 4.60 (d, *J* = 12.0 Hz, 6 H), 4.54–4.46 (m, 40 H), 4.42 (d, *J* = 7.6 Hz, 12 H), 4.38 (d, *J* = 3.2 Hz, 12 H), 4.36–4.32 (m, 12 H), 4.27 (d, *J* = 9.6 Hz, 4 H), 4.19 (d, *J* = 11.6 Hz, 4 H), 4.06–3.95 (m, 80 H), 3.67 (d, *J* = 12.0 Hz, 2 H), 3.61 (d, *J* = 11.6 Hz, 5 H), 3.58–3.53 (m, 12 H), 3.48–3.44 (m, 18 H), 3.20 (dd, *J* = 3.2, 10.0 Hz, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.5, 139.40, 139.37, 139.34, 139.2, 139.1, 138.9, 138.54, 138.51, 138.46, 138.43, 138.37, 138.34, 138.12, 138.05, 138.02, 137.96, 134.6, 129.9, 129.1, 128.7, 128.5, 128.4, 128.34, 128.27, 128.20, 128.10, 128.05, 127.99, 127.94, 127.90, 127.76, 127.72, 127.68, 127.55, 127.50, 127.4, 127.33, 127.28, 127.2, 127.1, 127.0, 126.9, 98.7, 98.5, 81.8, 81.1, 81.0, 80.7, 80.4, 79.8, 79.7, 79.5, 79.1, 79.0, 78.91, 78.89, 78.84, 78.81, 78.7, 78.21, 78.19, 76.0, 75.5, 75.43, 75.42, 75.3, 74.2, 73.9, 73.5, 73.42, 73.38, 73.0, 72.9, 72.83, 72.79, 72.7, 72.6, 72.5, 71.71, 71.68, 71.6, 71.5, 69.4 ppm. HRMS (MALDI-TOF): *m*/*z* calcd. for $C_{518}H_{588}N_6O_{58}$ [M + Na]$^+$ 8432.7197; found 8433.8854; calcd. for [M + K]$^+$ 8448.6937; found 8449.4727. R_f 0.23 (EtOAc/pentane; 1:2.5).

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-($2^{A–G}$,$3^{A–G}$,$6^{B–G}$-heptadeca-*O*-benzyl-β-cyclodextrin-6^A-oxy)-1,2,3-triazolyl-4-methyl]}hexadeca-*O*-benzyl-α-cyclodextrin (16): CuI (32.9 mg, 0.17 mmol) and DIPEA (0.05 mL, 0.26 mmol) were added to a solution of **4β** (0.54 g, 0.18 mmol) and **2β** (0.25 g, 0.09 mmol) in MeCN (2 mL) and EtOAc (2 mL) and the resulting mixture was stirred until TLC showed no diazide. The reaction was then quenched by adding water (20 mL) and a saturated aqueous solution of NH_4Cl (10 mL). The aqueous layer was extracted three times with EtOAc, the combined organic phases were washed with brine, dried with $MgSO_4$, and the solvent was removed in vacuo. The resulting oil was purified by flash column chromatography (EtOAc/pentane; 1:4 → 1:2) to give **16** as a white foam in a 1:1 mixture of isomers (0.58 g, 0.07 mmol, 73%); m.p. 81.8–88.3 °C. ^{1}H NMR ($CDCl_3$): δ = 7.29 (s, 4 H), 7.21–7.05 (m, 590 H, Ph), 5.65 (s, 1 H), 5.56 (s, 1 H), 5.43 (s, 1 H), 5.31 (s, 1 H), 5.26 (d, *J* = 15.6 Hz, 7 H), 5.19–4.92 (m, 63 H), 4.76–4.72 (m, 28 H), 4.64 (d, *J* = 9.6 Hz, 6 H), 4.58 (d, *J* = 12.4 Hz, 3 H), 4.45–4.31 (m, 154 H), 4.22 (t, *J* = 11.2 Hz, 14 H), 4.03–3.96 (m, 175 H), 3.53 (t, *J* = 9.6 Hz, 28 H), 3.46–3.43 (m, 49 H), 3.20 (dd, *J* = 10.4, 19.2 Hz, 8 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 139.48, 139.45, 139.41, 138.56, 138.52, 138.48, 138.44, 138.37, 128.8, 128.6, 128.4, 128.3, 128.2, 128.12, 128.10, 128.06, 127.99, 127.94, 127.90, 127.88, 127.7, 127.6, 127.5, 127.32, 127.25, 127.19, 127.0, 98.7, 98.5, 81.1, 81.0, 79.04, 78.97, 78.93, 78.91, 78.86, 78.78, 78.73, 78.1, 75.54, 75.46, 75.1, 73.5, 73.43, 73.39, 72.90, 72.85, 72.7, 72.6, 71.7, 71.7, 69.5, 69.44, 69.41 ppm. HRMS (MALDI-TOF): *m*/*z* calcd. for $C_{545}H_{566}N_6O_{103}$ [M + Na]$^+$ 8864.9134; found 8865.4036; calcd. for [M + K]$^+$ 8880.8873; found 8881.3359. R_f 0.23 (EtOAc/pentane; 1:2.5).

Hydrogenolysis of Trimers. General Procedure: Trimer was dissolved in CH_2Cl_2/EtOH (1:1), Raney nickel (3 mL) was added and the resulting mixture was stirred for 1 h before being filtering through a cotton plug. The filtrate was evaporated in vacuo and dissolved in 2-methoxyethanol, then $Pd(OH)_2$ was added and 1 atm of hydrogen gas applied, and finally a few drops of TFA were added. The progress of the reaction was monitored by MALDI-TOF MS and took from 7 to 30 d to go to completion. The reaction mixture was filtered through a silica plug and the solvents were evaporated. The white solid residue was freeze-dried to give a powder.

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-(α-cyclodextrin-6-oxy)-1,2,3-triazolyl-4-methyl]}-α-cyclodextrin (17): ^{1}H NMR (D_2O): δ = 8.36 (s, 2 H), 5.17 (d, *J* = 3.2 Hz, 4 H), 5.05 (s, 18 H), 4.09 (d, *J* = 9.2 Hz, 4 H), 3.98–3.96 (m, 22 H), 3.89–3.87 (m, 30 H), 3.83 (s, 32 H), 3.66–3.60 (m, 40 H), 3.58–3.55 (m, 22 H), 3.26 (d, *J* = 11.2 Hz, 2 H), 2.80 (d, *J* = 11.2 Hz, 2 H) ppm. ^{13}C NMR (D_2O): δ = 110.0, 95.1, 94.4, 81.7, 73.5, 72.2, 71.9, 65.5, 62.7, 60.6, 60.5, 59.6, 58.2, 57.2, 51.7 ppm. HRMS (MALDI-TOF): *m*/*z* calcd. for $C_{114}H_{182}N_6O_{88}$ [M + Na^+ 3065.9849; found 3064.8098; calcd. for [M + K]$^+$ 3081.9588; found 3080.7580. Elution time: 16.97 min [1 mL min^{-1}, 60 min, 5–90% MeCN in H_2O (0.1% TFA)].

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-(α-cyclodextrin-6-oxy)-1,2,3-triazolyl-4-methyl]}-β-cyclodextrin (18): ^{1}H NMR (D_2O): δ = 8.33 (s, 1 H), 7.99 (s, 3 H), 5.07 (s, 5 H), 4.95 (d, *J* = 3.2 Hz, 46 H), 4.90–4.89 (m, 5 H), 3.82 (t, *J* = 3.6 Hz, 53 H), 3.75–3.72 (m, 88 H), 3.61 (d, *J* = 4.4 Hz, 8 H), 3.60–3.59 (m, 7 H), 3.58 (d, *J* = 0.8 Hz, 4 H), 3.575 (d, *J* = 0.8 Hz, 2 H), 3.57 (d, *J* = 2.4 Hz, 4 H), 3.56 (d, *J* = 1.2 Hz, 4 H), 3.54 (s, 23 H), 3.52–3.47 (m, 74 H), 2.82 (d, *J* = 10.4 Hz, 5 H) ppm. ^{13}C NMR (D_2O): δ = 101.6, 96.0, 95.7, 81.5, 73.5, 73.3, 72.2, 71.9, 69.8, 61.7, 60.6 ppm. HRMS (MALDI-TOF): *m*/*z* calcd. for $C_{120}H_{192}N_6O_{93}$ [M + Na]$^+$ 3228.0377; found 3228.7444; calcd. for [M + K]$^+$ 3244.0116; found 3244.1412. Elution time: 17.41, 18.03 min [1 mL min^{-1}, 60 min, 5–90% MeCN in H_2O (0.1% TFA)].

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-(β-cyclodextrin-6-oxy)-1,2,3-triazolyl-4-methyl]}-α-cyclodextrin (19): ^{1}H NMR (D_2O): δ = 8.13 (s, 2 H), 5.23 (s, 2 H), 5.17 (d, *J* = 14.0 Hz, 2 H), 5.10 (s, 9 H), 5.07 (s, 4 H), 5.01 (d, *J* = 7.6 Hz, 3 H), 4.09–4.03 (m, 7 H), 3.97 (d, *J* = 9.2 Hz, 23 H), 3.90 (s, 20 H), 3.87 (s, 16 H), 3.84–3.73 (m, 11 H), 3.68 (d, *J* = 10.8 Hz, 18 H), 3.62 (d, *J* = 9.2 Hz, 18 H), 3.60–3.54 (m, 7 H), 3.20 (d, *J* = 13.2 Hz, 2 H), 2.98 (d, *J* = 12.0 Hz, 2 H) ppm. ^{13}C NMR (D_2O): δ = 110.0, 95.3, 95.2, 82.1, 73.4, 72.4, 72.3, 71.9, 71.24, 71.20, 69.5, 69.3, 66.8, 64.1, 62.8, 60.7, 60.6, 58.2 ppm. HRMS (MALDI-TOF): *m*/*z* calcd. for $C_{126}H_{202}N_6O_{98}$ [M + Na]$^+$ 3390.0905; found 3386.0380; calcd. for [M + K]$^+$ 3406.0644; found 3403.4028. Elution time: 16.38 min [1 mL min^{-1}, 60 min, 5–90% MeCN in H_2O (0.1% TFA)].

6^A,6^D-Dideoxy-6^A,6^D-bis{*N*-[4′-(β-cyclodextrin-6-oxy)-1,2,3-triazolyl-4-methyl]}-β-cyclodextrin (20): ^{1}H NMR (D_2O): δ = 8.33 (s, 2 H), 8.00 (s, 2 H), 5.08 (s, 3 H), 4.95 (s, 27 H), 4.89 (s, 6 H), 4.85 (s, 2 H), 3.84 (t, *J* = 9.2 Hz, 37 H), 3.76 (s, 50 H), 3.72 (s, 35 H), 3.63–3.62 (m, 3 H), 3.61 (s, 4 H), 3.60 (d, *J* = 1.2 Hz, 5 H), 3.59 (t, *J* = 1.2 Hz, 4 H), 3.57 (d, *J* = 2.0 Hz, 5 H), 3.56 (t, *J* = 1.2 Hz, 6 H), 3.54 (d, *J* = 1.6 Hz, 18 H), 3.53 (t, *J* = 1.2 Hz, 14 H), 3.52–3.51 (m, 22 H), 3.49–3.45 (m, 43 H), 3.27 (d, *J* = 1.2 Hz, 12 H),

3.25 (d, J = 1.2 Hz, 2 H), 3.14 (s, 4 H) ppm. ^{13}C NMR (D_2O): δ = 102.1, 102.0, 81.3, 81.2, 73.23, 73.15, 72.21, 72.15, 72.0, 71.9, 71.6, 71.3, 69.5, 62.7, 60.6, 60.4, 58.2 ppm. HRMS (MALDI-TOF): *m/z* calcd. for $C_{132}H_{212}N_6O_{103}$ $[M + Na]^+$ 3552.1434; found 3557.6064; $[M + K]^+$ 3568.1173; found 3573.4937. Elution time: 12.86, 13.69 min [1 mL min^{-1}, 60 min, 5–90% MeCN in H_2O (0.1% TFA)].

Peptide Synthesis. General Procedure: The peptides were automatically synthesized by utilizing Fmoc solid-phase peptide synthesis on a Rink-amide resin. Couplings were performed on a Liberty automated microwave peptide synthesizer with an in situ Fmoc amino acid activation protocol with HBTU/DIPEA. Fmoc deprotection was achieved by treatment with 20% piperidine in DMF. After final deprotection the peptidyl resin was transferred to a filtration tube, washed several times with CH_2Cl_2, and then shrunk in MeOH for 30 min, and left overnight in a desiccator containing KOH (s). Cleavage was achieved by adding TFA/TIS/water (5 mL; 95:2.5:2.5 v/v) and swirling for 1 h, then filtering. The peptide was isolated by evaporation and subsequent freeze-drying. The peptides were dissolved in 20% MeCN in water and preparative purification achieved by RP-HPLC on an Agilent 1100 system with a Vydac column (C8, 208TP-510) 5–40% MeCN in milliQ H_2O (0.1% TFA).

FGGGFGGGF: ^{1}H NMR (D_2O): δ = 7.65–7.49 (m, 15 H, Ph), 4.50 (t, J = 7.2 Hz, 1 H), 4.24 (d, J = 16.8 Hz, 1 H), 4.17 (d, J = 16.8 Hz, 1 H), 4.14 (d, J = 2.8 Hz, 2 H), 4.11 (d, J = 2.8 Hz, 2 H), 4.09 (d, J = 2.8 Hz, 2 H), 4.06 (dd, J = 2.8, 6.4 Hz, 2 H), 3.45–3.38 (m, 4 H), 3.24 (dd, J = 8.8, 14.8 Hz, 1 H), 3.20 (dd, J = 9.2, 14.0 Hz, 1 H), 2.28 (qv, J = 2.4 Hz, 4 H) ppm. HRMS: *m/z* calcd. for $C_{39}H_{48}N_{10}O_9$ $[M + Na]^+$ 823.3503; found 823.3509. Elution time: 13.89 min.

FGGGNalGGGF: ^{1}H NMR (D_2O): δ = 8.27–8.24 (m, 3 H), 8.12 (d, J = 8.8 Hz, 1 H), 7.91–7.89 (m, 2 H), 7.81 (t, J = 8.0 Hz, 1 H), 7.76–7.62 (m, 10 H), 5.07 (dd, J = 7.2, 15.2 Hz, 1 H), 4.91 (dd, J = 5.6, 8.0 Hz, 1 H), 4.60 (t, J = 7.2 Hz, 1 H), 4.32–4.07 (m, 12 H), 3.74–3.68 (m, 1 H), 3.58–3.51 (m, 4 H), 3.31 (dd, J = 9.6, 13.6 Hz, 1 H) ppm. HRMS: *m/z* calcd. for $C_{43}H_{50}N_{10}O_9$ $[M + H]^+$ 851.3840; found 851.3840. Elution time: 17.41 min.

FGGGYGGGF: ^{1}H NMR (CD_3OD): δ = 7.37–7.26 (m, 10 H), 7.05 (d, J = 8.4 Hz, 2 H), 6.70 (d, J = 8.4 Hz, 2 H), 4.59 (dd, J = 5.2, 9.2 Hz, 1 H), 4.41 (dd, J = 6.0, 6.4 Hz, 1 H), 4.16 (dd, J = 6.4, 8.4 Hz, 1 H), 4.04 (d, J = 8.8 Hz, 1 H), 3.95–3.79 (m, 11 H), 3.27 (dd, J = 6.0, 14.4 Hz, 1 H), 3.19 (dd, J = 5.2,14.0 Hz, 1 H), 3.05 (dd, J = 8.4, 14.0 Hz, 2 H), 2.93 (dd, J = 9.6, 14.0 Hz, 1 H), 2.90 (dd, J = 8.4, 13.6 Hz, 1 H) ppm. HRMS: *m/z* calcd. for $C_{39}H_{48}N_{10}O_{10}$ $[M + Na]^+$ 839.3453; found 839.3533. Elution time: 12.94 min.

FGGGWGGGF: ^{1}H NMR (CD_3OD): δ = 7.56 (d, J = 8.0 Hz, 1 H), 7.36–7.25 (m, 12 H), 7.15 (s, 1 H), 7.09 (dt, J = 0.8, 8.0 Hz, 1 H), 7.01 (dt, J = 0.8, 8.0 Hz, 1 H), 4.62–4.52 (m, 2 H), 4.15 (dd, J = 6.0, 8.0 Hz, 1 H), 4.04 (dd, J = 6.0, 16.4 Hz, 1 H), 3.21 (dd, J = 4.0, 4.8 Hz, 1 H), 3.17 (dd, 1 H) 3.05 (dd, J = 8.4, 14.0 Hz, 1 H), 2.92 (dd, 1, 9.6, 14.0 Hz,) ppm. HRMS: *m/z* calcd. for $C_{41}H_{49}N_{11}O_9$ $[M + Na]^+$ 862.3612; found 862.3554. Elution time: 15.46 min.

FGGFGGF: ^{1}H NMR (CD_3OD): δ = 7.37–7.20 (m, 15 H), 4.57 (t, J = 4.8 Hz, 1 H), 4.55 (t, J = 5.2 Hz, 1 H), 4.15 (dd, J = 6.4, 8.0 Hz, 1 H), 3.97 (d, J = 16.0 Hz, 1 H), 3.90 (d, J = 3.6 Hz, 1 H), 3.87 (d, J = 5.6 Hz, 1 H), 3.84 (d, J = 8.0 Hz, 1 H), 3.79 (s, 2 H), 3.74 (d, J = 1.2 Hz, 2 H), 3.68 (d, J = 10.8 Hz, 1 H), 3.27 (dd, J = 8.0, 14.0 Hz, 1 H), 3.22 (dd, J = 2.8, 8.0 Hz, 1 H), 3.18 (dd, J = 6.4, 8.4 Hz, 1 H), 3.06 (dd, J = 8.0, 14.0 Hz, 1 H), 3.01 (dd, J = 5.6, 8.8 Hz, 1 H), 2.98 (dd, J = 6.4, 9.6 Hz, 1 H) ppm. HRMS: *m/z* calcd. for $C_{35}H_{42}N_8O_7$ $[M + Na]^+$ 709.3074; found 709.2978. Elution time: 16.05 min.

GGGF: ^{1}H NMR (D_2O/CD_3OD): δ = 7.47–7.33 (m, 5 H, Ph), 4.68 (dd, J = 6.0, 6.4 Hz, 1 H), 3.97 (d, J = 16.4 Hz, 1 H), 3.90 (d, J = 17.2 Hz, 1 H), 3.75 (dd, J = 16.4, 17.2 Hz, 2 H), 3.29 (s, 2 H), 3.25 (dd, J = 6.4, 14.0 Hz, 1 H), 3.16 (dd, J = 5.6, 14.8 Hz, 1 H) ppm. HRMS: *m/z* calcd. for $C_{15}H_{21}N_5O_4$ $[M + Na]^+$ 358.1491; found 358.1479. Elution time: 14.52 min.

Determination of Association Constants by Plasmon Surface Resonance (SPR): A manually operated BIAcore X® instrument and standard CM5® sensor chips were used. The peptides were covalently attached to the chip surface by standard amine coupling reactions in 10 mM acetate buffer adjusted to pH 4.6 by addition of HCl.[25] Experiments were carried out in 10 mM phosphate buffer at pH 4.6 with various concentrations of cyclodextrins ranging from 5 to 1000 μM.

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[1] H. Chen, W. S. Weiner, A. D. Hamilton, *Curr. Opin. Chem. Biol.* **1997**, *1*, 458–466.
[2] H. J. Schneider, A. K. Yatsimirsky, *Chem. Soc. Rev.* **2008**, *37*, 263–277.
[3] G. Arena, A. Casnati, A. Contino, A. Magrì, F. Sansone, D. Sciotto, R. Ungaro, *Org. Biomol. Chem.* **2006**, *4*, 243–249.
[4] K. D. Shimizu, J. Rebek Jr., *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 12403–12407.
[5] C. S. Wilcox, M. D. Cowart, *Carbohydr. Res.* **1987**, *171*, 141–160.
[6] Peptoids are membrane-active polypeptides derived from fungal sources, see: J. K. Chugh, B. A. Wallace, *Biochem. Soc. Trans.* **2001**, *29*, 565–570.
[7] J. Szetli, *Chem. Rev.* **1998**, *98*, 1743–1753.
[8] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875–1917.
[9] K. Sasaki, M. Nagasaka, Y. Kuroda, *Chem. Commun.* **2001**, *24*, 2630–31.
[10] a) Md. A. Hossain, H.-J. Schneider, *J. Am. Chem. Soc.* **1998**, *120*, 11208–11209; b) M. Kruppa, C. Mandl, S. Miltschitzky, B. König, *J. Am. Chem. Soc.* **2005**, *127*, 3362–3365; c) M. E. Bush, N. D. Bouley, A. R. Urbach, *J. Am. Chem. Soc.* **2005**, *127*, 14511–14517; d) M. Sirish, H.-J. Schneider, *Chem. Commun.* **1999**, 907–908; e) H.-J. Schneider, *Angew. Chem.* **2009**, *121*, 3982; *Angew. Chem. Int. Ed.* **2009**, *48*, 3924–3977.
[11] Recently click chemistry has been extensively used to attach functional groups to cyclodextrins or connect cyclodextrins to each other, see: a) K. Ohtsuka, S. Shinobu, S. Watanabe, K. Komizo, S. Takenaka, *Nucleic Acids Symp. Ser.* **2008**, *52*, 717–718; b) M. Mourer, F. Hapiot, E. Monflier, S. Menuel, *Tetrahedron* **2008**, *64*, 7159–7163; c) M. Mourer, F. Hapiot, S. Tilloy, E. Monflier, S. Menuel, *Eur. J. Org. Chem.* **2008**, 5723–5730; d) M. Munteanu, C. Soowhan, H. Ritter, *J. Inclusion Phenom. Macrocyclic Chem.* **2008**, *62*, 197–202; e) S. Srinivasachari, K. M. Fichter, T. Reineke, *J. Am. Chem. Soc.* **2008**, *130*, 4618–4627; f) Y. Wang, Y. Xiao, T. Tan, S.-C. Ng, *Tetrahedron Lett.* **2008**, *49*, 5190–5191; g) J. M. Bryson, W.-J. Chu, J.-H. Lee, T. M. Reineke, *Bioconjugate Chem.* **2008**, *19*, 1505–1509.
[12] A paper using the same assembly methodology as that used herein appeared recently, see: G. K. Rawal, P. Zhang, C.-C. Ling, *Org. Lett.* **2010**, *12*, 3096–3099.
[13] J. Bjerre, T. H. Fenger, L. G. Marinescu, M. Bols, *Eur. J. Org. Chem.* **2007**, 704–710.

[14] T. Lecourt, A. Herault, A. J. Pearce, M. Sollogoub, P. Sinaÿ, *Chem. Eur. J.* **2004**, *10*, 2960–2971.
[15] F. Ortega-Caballero, J. Bjerre, L. S. Laustsen, M. Bols, *J. Org. Chem.* **2005**, *70*, 7217–7226.
[16] D. Lesur, A. Gassama, V. Moreau, S. D. Pilard, F. Jedaïni-Pilard, *Carbohydr. Res.* **2005**, *340*, 1225–1231.
[17] V. O. Rodionov, V. V. Fokin, M. G. Finn, *Angew. Chem.* **2005**, *117*, 2250; *Angew. Chem. Int. Ed.* **2005**, *44*, 2210–2215.
[18] M. Ahlquist, V. V. Fokin, *Organometallics* **2007**, *26*, 4389–4391.
[19] S. Hotha, S. Kashyap, *J. Org. Chem.* **2006**, *71*, 364–367.
[20] S. Kamijo, T. Jin, Z. Huo, Y. Yamamoto, *J. Org. Chem.* **2004**, *69*, 2386–2393.
[21] R. E. Looper, D. Pizzirani, S. L. Schreiber, *Org. Lett.* **2006**, *8*, 206–2066.
[22] V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, *1*, 51–68.
[23] K. D. Bodine, D. Y. Gin, M. S. Gin, *Org. Lett.* **2005**, *7*, 4479–4482.
[24] S. Menuel, N. Azaroual, D. Landy, N. Six, F. Hapiot, E. Monflier, *Chem. Eur. J.* **2011**, *17*, 3949–3955.
[25] F. A. Tanious, B. Nguyen, W. D. Wilson, *Methods Cell Biol.* **2008**, *84*, 53–77.
[26] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875–1917.
[27] T. Lecourt, A. Herault, A. J. Pearce, M. Sollogoub, P. Sinaÿ, *Chem. Eur. J.* **2004**, *10*, 2960–2971.

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