Substantial Spatial Flexibility and Hydrogen Bonding within the Catalysis Exerted by Cyclodextrin Artificial Glycosidases

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Herein we report the synthesis of a novel $7^A,7^D$-dicyanohydrin-β-cyclodextrin that catalyzes the hydrolysis of aryl glycosides with up to 5500 times rate increase (k_{cat}/k_{uncat}), functioning as a glycosidase enzyme mimic. For all glycoside substrates tested at 50 mM phosphate buffer this catalysis is superior to previously reported results for $6^A,6^D$-dicyanohydrin cyclodextrin (CD) artificial glycosidases, i.e. analogues which have their catalytic group one carbon atom closer to the cyclodextrin cavity. This provides proof of substantial flexibility within the catalysis exerted by these CD chemzymes. A series of permethylated mono- and dicyanohydrin α- and β-CDs were also synthesized, and these showed more modest catalytic rate enhancements of up to 110 times (10 % catalysis rate, relative to non-methylated analogues), implying that the permethylation blocks or hampers catalytically important binding between the saccharide part of the substrate and the CD. For comparison, the permethylated $6^A,6^D$-dicarboxylic acid β-CD was also synthesized and afforded 25 % activity (up to 250 times rate increase) relative to the nonpermethylated $6^A,6^D$-dicarboxylic acid β-CD. This suggests that the catalytic effect of the polar interactions of the ionized carboxylate entity is less dependent on the substrate position. These findings afford new information on the scopes and boundaries for CD artificial glycosidase catalysis, and the spatial flexibility discovered fosters optimism for future advances and discoveries within the field of artificial enzymes.

Introduction

Catalysis is an art mastered to perfection by natural enzymes. These display magnificent rate enhancements (up to 10^{19}) and unmatched selectivity in their facilitation of chemical processes.[1] This impressive feat holds many possible applications and possibilities for whom understands it, and can mimic it.[2,3] The development of artificial enzymes is an interesting yet challenging task,[4] but through the use of modified CDs as macromolecular hosts,[5] we have recently seen the appearance of some of the first glycosidase mimics.[6] The cyclodextrins are cyclic α(1–4)glucopyranosides, i.e. circular structures comprised of 6 (for α-CD) or 7 (β-CD) alphabetically denominated glucose units which are connected in a ring, forming a cone-shaped macrostructure with an upper (primary) and lower (secondary) rim.[7] They are well-suited for supramolecular catalysis purposes, owing to the ability of the predominantly nonpolar CD cavity to selectively bind small lipophilic substrates, whilst the polar CD exterior makes the complex soluble in aqueous medium. Our efforts have been concentrated on performing selective chemical manipulations on the primary rim of α- and β-CD, equipping these with catalytically active groups that can facilitate the hydrolysis of the glycosidic bonds of bound glycoside substrates. Whilst most natural enzymes are grand, bulky peptide assemblies with many motifs and regions that play no direct role in substrate binding and catalysis, our CD enzyme ensembles are almost solely comprised of pure binding site with attached catalytic groups. This gives the CD chemzymes a head start when it comes to catalytic power-to-mass ratio. Albeit we have yet to see CD artificial enzymes able to equally compete with the impressive catalytic power of natural enzymes, this fuels incitement to further explore and research the field of CDs as biomimetic catalysts.[8,9]

The inspiration for the design of the CD glycosidases came from the world of natural glycosidases which in their catalysis of glycosidic bond hydrolysis typically encompass two catalytically active carboxylate groups in their active site.[10,11] This acid/base-catalysis principle spawned the invention of firstly the carboxylate cyclodextrin glycosidases that were able to catalyze aryl glycoside bond hydrolysis reactions with rate enhancements of up to 1000 times (k_{cat}/k_{uncat}), following the enzyme-characteristic Michaelis–Menten rate law pattern[12,13] (see below). By obeying Michaelis–Menten kinetics, the chemzymes reveal themselves as enzyme-like mimics, displaying saturation kinetics, and not mere catalysts.

$$V_{cat} = \frac{V_{max}[S]}{K_M + [S]}$$

A true enzyme mimic should engage in binding of the substrate in the active site as a crucial constituent of the process of catalysis, which for CD chemzymes translates to

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substrate binding inside the cavity of the catalyzed rate-determining step of the catalyzed reaction. This binding criterion should be fulfilled for genuine enzyme-like catalysts, and the biomimetic CD chemzymes accordingly also fulfil this demand in their catalysis. Cavity substrate binding has previously been shown for CD chemzymes, in that the addition of an inhibitor which competes with the substrate for available cavity space, effectively lowers catalysis rate. Substrate recognition and selectivity are also important hallmarks of enzyme-like catalysis, and the artificial glycosidase CDs show distinct substrate selectivity, acting like enzyme mimics also in this regard. Other cyclodextrins chemzymes that act as artificial glycosidases, are the trifluoromethyl alcohol CDs, but they do so with modest activity.[14] The greater breakthrough in CD artificial glycosidases came with the discovery of the CD cyanohydrins, affording up to 8000 times increase in reaction rate.[15–17] The CD cyanohydrins are the most effective CD glycosidases yet crafted, and interestingly they have even mastered catalysis of a range of natural toxic glucosides.[18] We here present the latest developments in CD cyanohydrins, whose catalysis surpasses previously reported results, and novel findings for permethylated cyanohydrin- and carboxylate-CD artificial glycosidases.

Results and Discussion

Probing the Catalytic Spatial Flexibility by Synthesis of $7^A,7^D$-Dicyanohydrin β-CD

The spatial boundaries of catalysis for CD cyanohydrins were explored, through synthesis of $7^A,7^D$-dicyanohydrin-β-CD, in which the cyanohydrin functionality is positioned one carbon atom further away from the cavity than in the known $6^A,6^D$-dicyanohydrin CDs. In the proposed mechanism for the catalyzed reaction, the electron-withdrawing effect of the nitrile group acidifies the cyanohydrin hydroxy, easing the donation of this alcohol proton to the substrate glycosidic oxygen, thereby facilitating bond cleavage (Figure 1, mechanism shown with the novel $7^A,7^D$-dicyanohydrin CD **13** herein reported). In the previously reported $6^A,6^D$-dicyanohydrin-β-CD, the cyanohydrin group is of the *gt*-(*R*)-conformation, with the hydroxy group conveniently pointing towards CD cavity and thereby being in vicinity of the bound substrate.

In matters of synthesis, aldehydes form straightforward precursors to cyanohydrins, via nucleophilic addition of cyanide. DIBAL-H reduction of the $6^A,6^D$-dinitrile β-CD **1**[15] to obtain a $7^A,7^D$-dialdehyde was initially attempted but this afforded the $7^A,7^D$-diamine **2** instead (Scheme 1).[19,20] Attempts to de-*O*-benzylate the $7^A,7^D$-diamine resulted in decomposition.

Instead, to obtain the $7^A,7^D$-dialdehyde-β-CD **5** a thioacetal-based approach was put to use.[21] From the $6^A,6^D$-diiodide-β-CD (**3**)[15] the $6^A,6^D$-dithiane-β-CD (**4**) was synthesized. Iodomethane/MeCN/NaHCO$_3$-type thioacetal deprotection of **4** was not effective, but a mercury-based deprotection afforded the $7^A,7^D$-dialdehyde-β-CD **5**
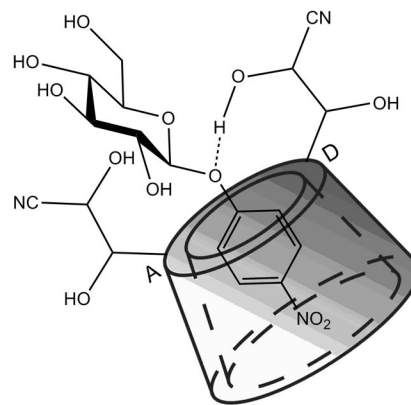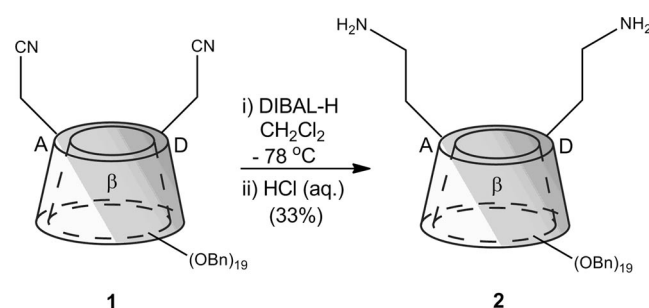


Figure 1. Proposed mechanism of cyanohydrin CD catalysis.

Scheme 1. Synthesis of $7^A,7^D$-diamine β-CD **2**.

in 44% yield.[22] Addition of cyanide gave rise to the benzyl-protected $7^A,7^D$-dicyanohydrin-β-CD **6**, but this compound was labile in the final de-*O*-benzylation step (Scheme 2).

A feasible route by which to achieve $7^A,7^D$-dicyanohydrin CDs proved to be dihydroxylation of the corresponding $5^A,5^D$-divinylnitrile CD. This divinylnitrile was obtained through Wittig addition of iodo-cyano-triphenylphosphorane to the $6^A,6^D$-dialdehyde CD (Scheme 3). Experiments on using either this Wittig type reagent vs. the corresponding Horner–Emmons type reagent diethyl cyanomethylphosphonate showed that the Horner–Emmons reagent afforded up to 85–90% bis(*E*)-diastereomer, but only the Wittig type reagent gave rise to the pure bis(*E*)-isomer product (Table 1). Consequently, the Wittig approach was chosen, affording the $5^A,5^D$-divinylnitrile CD.

In order to carry out the following olefin dihydroxylation reaction step in an asymmetric, enantioselective fashion, the Sharpless chiral dihydroxylation ligands α-mix (DHQ)$_2$PHAL or β-mix (DHQD)$_2$PHAL, were utilized under basic conditions (K$_2$CO$_3$) together with OsO$_4$[23,24] (Table 2). The two chiral ligands give rise to two facially different directions of approach for the *syn*-dihydroxylation to occur, and the relative stereochemistry of the 7^A- or the 7^D-position follows from this; it is the opposite stereogenicity of that of the adjacent 6^A- or 6^D-position [*rel*-($6^A R,6^D R$),($7^A S,7^D S$)]. The ligand-mediated induction of chirality is only efficient with *trans*-olefins, which were either the single or the major products of the Wittig ad-
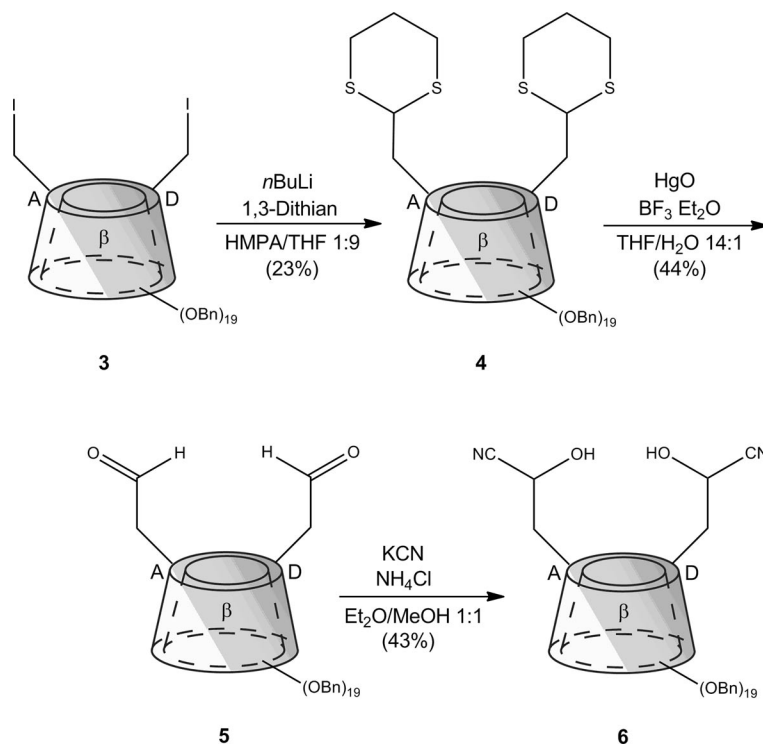
Scheme 2. Synthesis of nonadecabenzylated $7^A,7^D$-dicyanohydrin-β-CD **6**.

dition reaction, and thus it is only illustrated for the $5^A,5^D$-(E,E)-divinylnitrile CDs in Table 2. Different cooxidants such as $K_3Fe(CN)_6$ or $NaIO_4$ were used with or without $MeSO_2NH_2$ osmate ester hydrolysis aid, but in all cases the outcome was not fruitful.

The dihydroxylation reaction of the inactivated electron-poor alkene in the $5^A,5^D$-divinylnitrile CD was attempted in the absence of chiral ligand, using standard OsO_4 conditions with NMO as reoxidant, but this was not sufficient. Instead, an OsO_4 dihydroxylation procedure using citric acid was tried out, which by its acidic nature excludes the use of the basic Sharpless chiral ligands.[25] The citric acid protocol failed with use of $K_3Fe(CN)_6$ or NMO as cooxidants, but worked using the cooxidant $NaIO_4$. This way, dihydroxylation was effected successfully, affording the $7^A,7^D$-dicyanohydrins **9** and **12**, in 67% and 75% yield respectively. Due to the lack of chiral induction in the dihydroxylation reaction step, a mixture of $6^A,6^D$-stereisomers resulted; the OsO_4-catalyzed dihydroxylation reaction itself results in cis-type relative stereochemistry by its syn-nature of mechanism, but the facial direction of approach is not restricted in the absence of chiral ligand action. In the α-CD macrocycle, which is comprised of 6 glucose units, there is an axis of symmetry, making the mixed stereogenic center $6^A,6^D$ (S,R) and (R,S) enantiomers identical. This is not the case for the seven-membered β-CD macrocycle, wherein the $6^A,6^D$ (S,R) and (R,S) enantiomers are different isomer compounds. Therefore, including the two $6^A,6^D$ (S,S) and (R,R) configurations as well, a total of 3 isomerically different $6^A,6^D$-disubstituted α-CDs are the possible products from the dihydroxylation reaction step of $5^A,5^D$-(E,E)-di-

vinylnitrile α-CD, whereas 4 different isomer compounds of $6^A,6^D$-disubstituted β-CDs are possible from this reaction on β-CD **11** (Scheme 3). These considerations only include the dihydroxylation reaction products of pure $5^A,5^D$-(E,E)-divinylnitrile isomer CD starting materials; the portion of $5^A,5^D$-(Z,Z)-divinylnitrile present in the α-CD olefin compound **8** will upon dihydroxylation give rise to the opposite relative stereochemistry for the 6- and 7-adjacent positions [i.e. rel-($6^AR,6^DR$),($7^AR,7^DR$) for (Z,Z)-olefin] compared to that of the dihydroxylation reaction product of the $6^A,6^D$-(E,E)-divinylnitrile [rel-($6^AR,6^DR$),($7^AS,7^DS$)]. And the $6^A,6^D$-position mixed stereogenicity α-CD olefin [i.e. (E,Z) = (Z,E)] will upon dihydroxylation bring about the mixed combination product, rel-($6^AR,6^DR$),($7^AS,7^DR$) = rel-($6^AR, 6^DR$),($7^AR,7^DR$); consequently, scrambling of stereochemistry of all $6^A,6^D,7^A,7^D$ asymmetric centers is possible for the α-CD dihydroxylation product **9** and **10** different isomers of this product are therefore possible. The reaction sequence, including the dihydroxylation reaction step, was initially pursued with α-CD, but the final de-O-benzylation step was difficult to realize and caused decomposition of the $7^A,7^D$-dicyanohydrin-α-CD **9**.

The reaction sequence was then undertaken with isomerically pure $5^A,5^D$-(E,E)-divinylnitrile β-CD, and the $7^A,7^D$-dicyanohydrin **12** was stable during the deprotection reaction, yielding the desired unprotected $7^A,7^D$-dicyanohydrin-β-CD **13**.

For comparison purposes, the de-O-benzylation reactions of both the α-CD and β-CD $5^A,5^D$-divinylnitriles **8** and **11** were attempted, but in both cases this led to decomposition of the compounds.
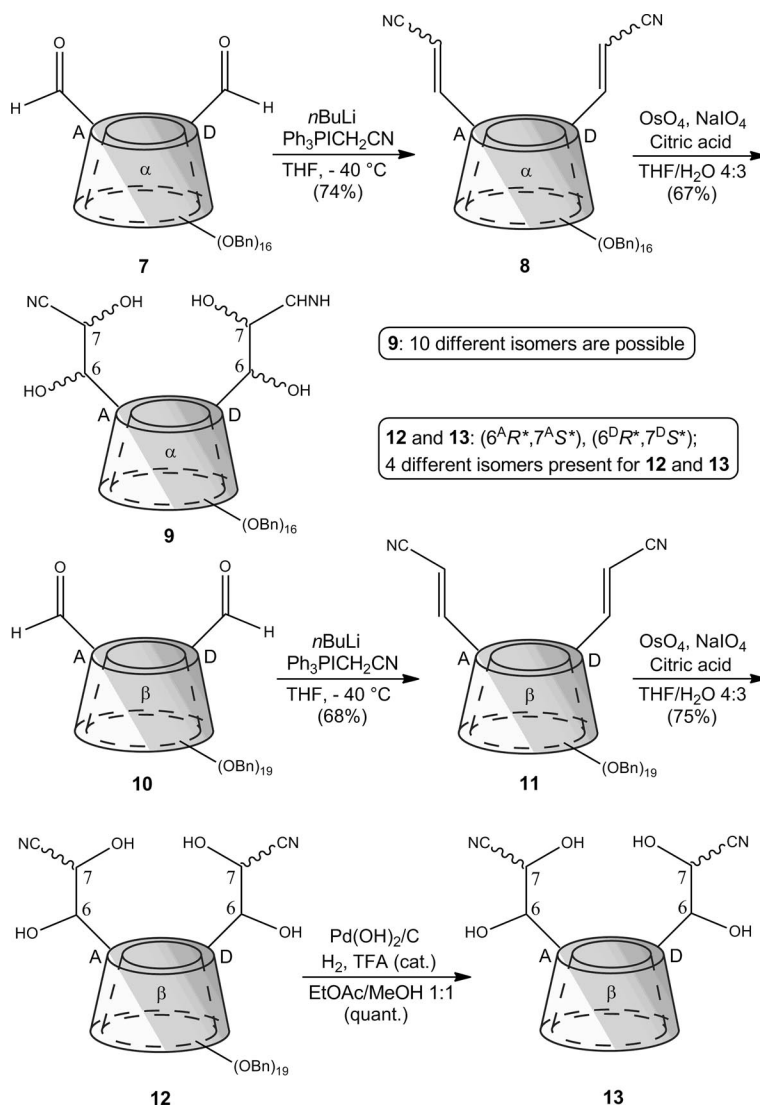
Scheme 3. Syntheses of α- and β-CD 7ᴬ,ᴰ-dicyanohydrins.

Table 1. Effect of the nature of the nucleophile and base on yield and isomeric ratio of 5ᴬ,5ᴰ-divinylnitrile CD.

Nucleophile	Base	Reaction time	Temp. [°C]	% Yield	% of bis(E)-isomer
(OEt)$_2$POCH$_2$CN 10 equiv.	NaH 10 equiv.	0.5 h	–40	44	85–90
(OEt)$_2$POCH$_2$CN 12 equiv.	nBuLi 5.8 equiv.	1.4 h	–40	59	85–90
(OEt)$_2$POCH$_2$CN 10 equiv.	nBuLi 4.8 equiv.	0.5 h	0	80	85–90
Ph$_3$PICH$_2$CN 9 equiv.	nBuLi 4.9 equiv.	1.5 h	–40	49	100
Ph$_3$PICH$_2$CN 10 equiv.	NaH 10 equiv.	7 h	–40	68	100

Catalysis Assay Data for 7ᴬ,7ᴰ-Dicyanohydrin β-CD

The 7ᴬ,7ᴰ-dicyanohydrin-β-CD 13 was tested for catalysis of p-nitrophenyl glycoside hydrolysis in phosphate buffer at 59 °C; the results are listed in Table 3. The catalyzed reactions all obey the Michaelis–Menten kinetic rate law, show

substrate recognition and selectivity, and require binding of the substrate inside the CD cavity. All these features are in accordance with the observations done for natural enzymatic catalysis, implying that the CDs are functioning as true glycosidase mimics. For inhibition testing, the presence of the CD cavity binding compound β-naphthalen sulfonate in the catalysis assay gave rise to a decrease in reaction rate in the CD-catalyzed reactions, through competition with the substrate for available CD cavity space. This is well in line with previous observations for CD chemzyme catalysis.
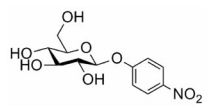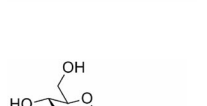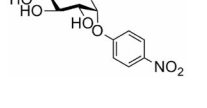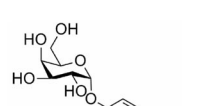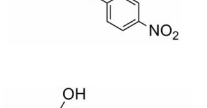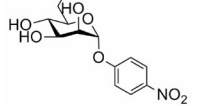
Table 3 illustrates that 7ᴬ,7ᴰ-dicyanohydrin-β-CD (13) catalyzes the hydrolysis reaction with rate enhancements (k_{cat}/k_{uncat}) up to 5500 times for p-nitrophenyl β-D-glucopyranoside. In all cases in 50 mM phosphate buffer, 13 is a more effective chemzyme than the previously studied analogue 6ᴬ,6ᴰ-dicyanohydrin-β-CD[15]. This provides proof of substantial flexibility within the catalysis, in that the catalytic cyanohydrin group will give rise to efficient catalysis regardless of whether it being in the 6ᴬ,6ᴰ- or 7ᴬ,7ᴰ-positions on the CD. In accordance with observations earlier

Table 2. Experiments of dihydroxylation reactions on $5^A,5^D$-divinylnitrile CD. In reactions with chiral ligand, $K_2OsO_2(OH)_4$ was used as OsO_4 source. The chiral induction is shown for (E)-divinylnitrile CD only; the reaction does not afford good chiral selectivity for cis-olefins.
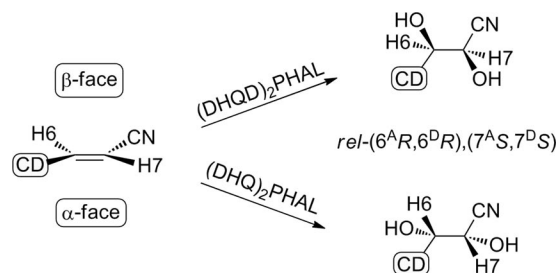
Acid or base	OsO_4	Chiral ligand	Cooxidant	$CH_3SO_2NH_2$	Reac. time	% Yield
K_2CO_3 14 equiv.	1 equiv.	$(DHQ)_2PHAL$ 5 mol-%	$K_3Fe(CN)_6$ 14 equiv.	2 equiv.	5 d	–
K_2CO_3 38 equiv.	1 equiv.	$(DHQD)_2PHAL$ 13 mol-%	$K_3Fe(CN)_6$ 38 equiv.	2 equiv.	4 d	–
K_2CO_3 35 equiv.	2 equiv.	$(DHQ)_2PHAL$ 6 mol-%	$K_3Fe(CN)_6$ 37 equiv.	–	2 d	–
K_2CO_3 31 equiv.	2 equiv.	$(DHQ)_2PHAL$ 10 mol-%	$K_3Fe(CN)_6$ 31 equiv.	–	5 d	–
K_2CO_3 14.3 equiv.	1.3 equiv.	$(DHQD)_2PHAL$ 3 mol-%	$K_3Fe(CN)_6$ 8.3 equiv.	–	7 d	–
–	0.2 equiv.	–	4.2 equiv. NMO	–	8 d	–
Citric acid 2 equiv.	0.8 equiv.	–	6 equiv. $K_3Fe(CN)_6$	–	7 d	–
Citric acid 2 equiv.	0.14 equiv.	–	6 equiv. NMO	–	6 d	–
Citric acid 2 equiv.	0.2–0.8 equiv.	–	77 equiv. $NaIO_4$ 4.2 equiv.	–	1–3 d	67–75

Table 3. Kinetic parameters for the hydrolysis of various glycosides in phosphate buffer at pH 8.0, 59 °C, catalyzed by $7^A,7^D$-dicyano-hydrin-β-CD (13). For k_{cat}/k_{uncat} ratios, k_{uncat} values from identical $6^A,6^D$-dicyanohydrin-β-CD studies were used.

Substrate	Phosphate (mM)	k_{cat} (10^{-5} s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
	50	6.50±1.26	2.75±2.69	3450±429
	500	12.10±0.94	6.53±1.56	5450±261
	500	9.31±1.98	4.24±3.08	5194±329
	50	5.76±0.44	3.44±0.90	1985±217
	50	2.69±0.28	1.55±0.46	399±43
	500	8.70±0.63	4.63±0.69	1352±147

made for CD cyanohydrins, some effect of phosphate buffer molarity can be seen but the correlation is not linear. The structure of the aglycon part of the substrate is of some importance and follows the trends noted in earlier studies, with the best substrates being the *p*-nitrophenyl glucopyranosides. The Michaelis–Menten constant K_m can be viewed as a measure of the binding strength of substrate to the CD, and in all cases the values obtained (1.5–6.5 mm) imply that the binding seems to be quite strong and follows those previously seen for CD chemzymes.

Attempted Truncation of Cyanohydrin Hydroxy

To further study the catalyzed reaction, efforts were made to truncate the cyanohydrin alcohol. According to the assumed mechanism of catalysis, this would strongly impede or even demolish catalysis. The $6^A,6^D$-dicyanohydrin groups in the β-CD **14** were acetylated in good yield,[26] but the de-*O*-benzylation of the product **15** brought about decomposition (Scheme 4).
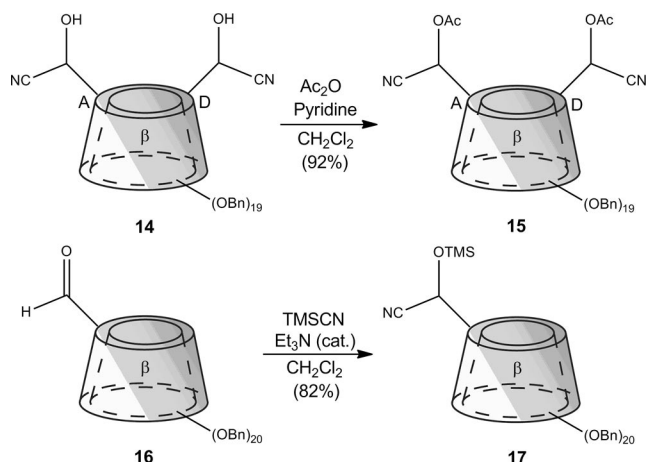
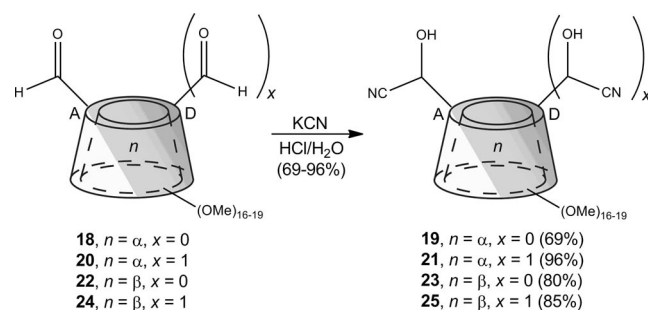Scheme 4. Syntheses of truncated β-CD cyanohydrins **15** and **17**.

Reactions aimed at achieving methylation of the cyanohydrin alcohol of **14**, utilizing either trimethylsilyldiazomethane, or iodomethane with sodium hydride, were not successful. As previously reported, methylation reactions using diazomethane in situ gave rise to a CD dimethylketone compound instead.[27] Masking the cyanohydrin alcohol via silylation, using TBDPSCl, failed, and the inherent tendency of the cyanohydrin functionality to decompose into aldehyde in the presence of base generally makes chemical manipulations of cyanohydrin groups difficult. A direct route of synthesis, viable for obtaining a TMS-truncated cyanohydrin, involved reaction of the 6-monoaldehyde-β-CD **16** with TMSCN; this afforded the isolated TMS-truncated cyanohydrin **17** in good yield (Scheme 4). Unfortunately, this product was not stable under de-*O*-benzylation reaction conditions.

In general, past experience has shown that in the palladium-catalyzed de-*O*-benzylations of modified CDs, even with generous amounts of acid present, CDs whose funtional groups contain free hydroxy- or carboxylate-groups

typically respond much better to de-*O*-benzylation reactions than those that lack any potential acidic protons. This is especially true for nitrile- or amine-functionalized CDs and the de-*O*-benzylation reactions described here pose no exception to this trend. Alternative de-*O*-benzylation reactions have been examined and tested in our group, but none have outperformed the H_2/Pd Scheme despite of its shortcomings. In the Pd-catalyzed hydrogenation, varying solvent system, hydrogen pressure, temperature etc. can sometimes be advantageous and is always investigated, but in difficult cases decomposition of CD or functional group can be most challenging to avoid.

### Permethylation of Cyanohydrin CD Artificial Glycosidases

One way to bypass the obstacle of tedious de-*O*-benzylations is the use of methylated CD derivatives that do not require a de-*O*-benzylation step at the end of their synthetic pathway.[28] Permethylation is in itself an interesting modification of the solubility, physical, and polar properties of the CD host. A number of methylated α- and β-CD cyanohydrins were synthesized through addition of cyanide to the corresponding 6-mono- or $6^A,6^D$-dialdehyde permethylated CDs.[27] TMSCN was not suited as cyanide source as it did only react slowly and the subsequent acid-catalyzed removal of TMS from the cyanohydrin alcohol caused breakdown of the cyanohydrin to aldehyde. KCN in combination with NH_4Cl afforded a messy reaction but when aq. HCl was used together with KCN, the desired cyanohydrin products **19**, **21**, **23**, and **25** were obtained (Scheme 5).

18, *n* = α, *x* = 0   **19**, *n* = α, *x* = 0 (69%)
20, *n* = α, *x* = 1   **21**, *n* = α, *x* = 1 (96%)
22, *n* = β, *x* = 0   **23**, *n* = β, *x* = 0 (80%)
24, *n* = β, *x* = 1   **25**, *n* = β, *x* = 1 (85%)

Scheme 5. Syntheses of methylated α- and β-CD mono- and dicyanohydrins **19**, **21**, **23**, and **25**.

Cyanohydrin Stereochemistry

The perbenzylated β-CD $6^A,6^D$-dicyanohydrin previously synthesized was proven to be exclusively of the (*R*,*R*)-cyanohydrin configuration, which would be the result of nucleophilic addition to the aldehyde of cyanide approaching from the outside of the CD (Scheme 6). The considerable steric hindrance resulting from perbenzylation makes this a plausible explanation following the Cram′s rule. This would also be the expected product based on the Felkin–Anh rule, i.e. if the aldehyde adopts the shown reactive conformation shown in Scheme 6, suited for incoming attack of the nucleophile. The nucleophile approaches with an incoming
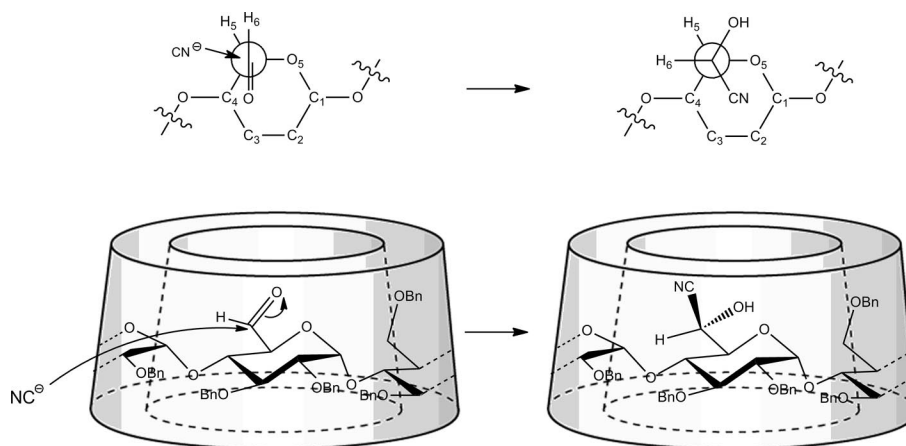
Scheme 6. Illustration of external approach of cyanide to afford (*R*)-isomer cyanohydrin CD in *gt*-conformation.

trajectory of approximately a 107° angle (the Bürgi–Dunitz angle) relative to the C=O bond; when the nucleophile also chooses the path shown, involving the least steric hindrance, the resulting product should be the *R*-isomer.

However, in case of the methylated CDs, the spatial crowding is much less than it is for the perbenzylated CDs, and both external and internal directions of approach of the incoming nucleophile might be envisaged; also, when having a more flexible macromolecule, the combined effects of both steric and electronic factors should be more intricate. Accordingly, for the methylated CDs, formation of both the cyanohydrin (*R*)- and (*S*)-isomers was observed. Other factors may also affect the steric outcome, e.g. a rapid dynamic equilibration of cyanohydrin and aldehyde under these conditions affording a mixture of isomer products. There may also be an effect arising from the conditions used, with this HCl/KCN protocol not being identical to the ammonium chloride/KCN and diethyl ether-employing procedure used for cyanide addition to the perbenzylated CD aldehydes.

In case of the 6-monocyanohydrins, two isomers were formed upon cyanide addition in approximate equal amounts and chromatographic separation of the two isomers was possible. For the $6^A,6^D$-dicyanohydrins, 4 different isomers for β-CD [(*R*,*R*), (*S*,*R*), (*R*,*S*) and (*S*,*S*)] were formed in one reaction step and partial isomeric separation of these by silica gel flash chromatography was achieved – the symmetry of α-CD makes the $6^A,6^D$ (*S*,*R*) and (*R*,*S*) isomers identical, and thus a total of 3 different isomer compounds are the result in this case. For $6^A,6^D$-dicyanohydrin permethylated CDs, the (*R*,*R*)-isomers were present only in trace amounts, whilst the (*R*,*S*)- and (*S*,*R*)-combined fraction was plentiful, and the (*S*,*S*)-isomer was also abundant, although to a lesser extent. The assigned stereochemistry arises from assumptions of isomeric configuration based on previous work in our group,[29] related to the observed polarity for the conformer at hand. As earlier described, the 6-position *tg*-conformation is unfavored due to steric repulsion of the C6 substituent with the neighboring glucopyranoside O5. Depending on isomer, the *gt* (for the

R-isomer) or *gg* (for the *S*-isomer) are appropriate conformations, in which the H6 gives rise to least amount of steric clash. In the *gt*-(*R*)-isomer, the cyanohydrin hydroxy is pointing inwards, towards the CD cavity, perfectly placed for exerting catalysis. Analogously, for the *gg*-(*S*)-isomer, the cyanohydrin hydroxy is pointing outwards, away from the CD cavity, which should be suboptimal for artificial enzyme catalysis.

Catalysis Assay Data for Permethylated CD Cyanohydrins

The different isomer fractions of the 4 permethylated cyanohydrin CDs were individually tested for artificial glycosidase activity on a number of different *p*-nitrophenyl glycosides (the *p*-nitrophenyl α- and β-galactosides, and the *p*-nitrophenyl α- and β-glucosides). The catalysis was followed by measuring the absorbance of *p*-nitrophenolate at 400 nm by UV/Vis, in 500 mM phosphate buffer, pH 8.0 at 59 °C. A modest Michaelis–Menten catalysis was found for the 6-(*R*)-monocyanohydrin α-CD and β-CD, both having the hydroxy in the cyanohydrin pointing towards the cavity. Due to the weak extent of catalysis affording a high error margin, it was not possible to accurately determine the K_m value for the β-CD, but for α-CD the K_m value of 5 mM is in line with previous findings for CD chemzymes. For the rest of the permethylated cyclodextrins tested, the error margin overpowered any potential feeble presence of Michaelis–Menten type catalytic activity (Table 4).

Table 4. Kinetic parameters for the permethylated 6^A-(*R*)-cyanohydrin α-CD (**19**) or β-CD (**21**) catalyzed hydrolysis of *p*-nitrophenyl-β-D-glucopyranoside in 500 mM phosphate buffer at pH 8.0, 59 °C.

6^A-(*R*)-cyanohydrin CD	k_{cat} [10^{-5} s^{-1}]	K_m [mM]	k_{cat}/k_{uncat}
α-CD (**19**)	0.12 ± 0.03	5.36 ± 2.75	113 ± 28
β-CD (**21**)	0.012 ± 0.006	not determined	6 ± 3

In the cases where no enzyme-like catalysis was found, the absorbance readings clearly revealed an increase in hydrolysis rate due to CD cyanohydrin, but which followed the simple rate law $V = (k_{cat} + k_{uncat})*[S]$. The (R,R)-isomers were not tested profoundly, as these were only present in trace amounts, so by analogy with the monocyanohydrin (R)-isomers being those equipped with catalytic activity, the remaining fractions would indeed be expected to perform poorer as chemzymes. However, the catalysis rate increase from the permethylated 6-monocyanohydrins is still 10 times lower than that for the previously studied non-methylated 6-monocyanohydrin β-CD. Evidently, permethylation dramatically decreases the enzyme-like catalytic potential of cyanohydrin CDs. We suspect that this may be due to a change in the binding mode between CD and substrate: Several different binding modes between CD and substrate is undoubtedly possible and the permethylation may prevent more catalytically active binding modes or favor less active ones.

Permethylation of Carboxylic Acid CD Artificial Glycosidases

The idea that permethylation may be hindering catalytically needed hydrogen bonding interactions between substrate and CD inspired us to examine this effect of permethylation in other CD artificial glycosidases. The $6^A,6^D$-dicarboxylic acid CDs were chosen as models of which to synthesize the permethylated analogues of. These unprotected carboxylate CD chemzymes can effect reaction rate en-

hancements of up to 1000 times for hydrolysis of aryl glycosides, and their mechanism of catalysis is proposed to involve electrostatic stabilization of the transition state by the carboxylate groups, with subsequent nucleophilic substitution with phosphate.[12,13] Two carboxylate catalytic groups are needed for chemzyme catalysis to occur; cyanohydrin CDs suffice having just one catalytic entity (Figure 2).

The permethylated analogues of the known 6-mono- and $6^A,6^D$-dicarboxylic acid β-CDs were synthesized by NaClO$_2$ oxidation of the corresponding permethylated CD aldehydes (Scheme 7).

Catalysis Assay Data for Permethylated CD Carboxylates

The permethylated carboxylic acid β-CDs were tested for catalysis of hydrolysis of *p*-nitrophenyl glycopyranosides in 500 mM phosphate buffer, pH 8.0 and 59 °C. For the permethylated 6-monocarboxylic acid β-CD, no catalysis was found. This shows that the catalytic requirements for the number of catalytic groups present for both unprotected and permethylated CD carboxylic acids are the same (Table 5).

The catalyzed reactions followed Michaelis–Menten kinetics steadily for the initial part of the reaction, but after one hour it started to follow the simple rate law $V = (k_{cat} + k_{uncat})*[S]$. Perhaps the change in catalysis could be due to either partial breakdown or aggregation of the CDs. Again, the small extent of catalytic activity makes precise K_m value determination challenging for some of the substrates; the K_m value of ca. 4 mM for the galactoside substrate is in line with previous findings. For the $6^A,6^D$-dicarboxylic acid β-CD, a catalysis with up to about 250 times rate enhancement was found. The observed catalysis rate is 4–10 times smaller for the permethylated dicarboxylate β-CD than for the unprotected dicarboxylate β-CD, showing that permethylation is not beneficial for catalysis. In this case too, the methylation may prevent or disfavor the catalytically active binding modes of the substrate.

Nevertheless, the reduction as a result of methylation is smaller in this case than for the methylated cyanohydrins **19** and **21**. This indicate that the cyanohydrins are more demanding than the dicarboxylic acids with respect to requirements for substrate position in the binding pocket. This is in agreement with the proposed mechanism of catalysis of both sets of chemzymes: For the cyanohydrins general acid catalysis has been proposed and this will only be
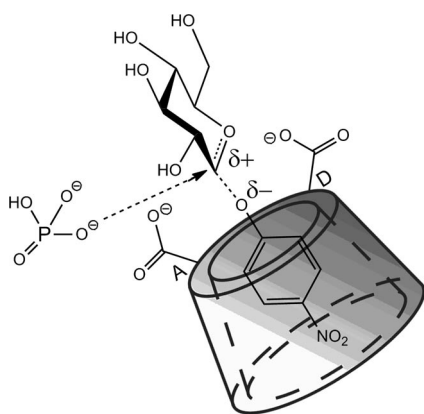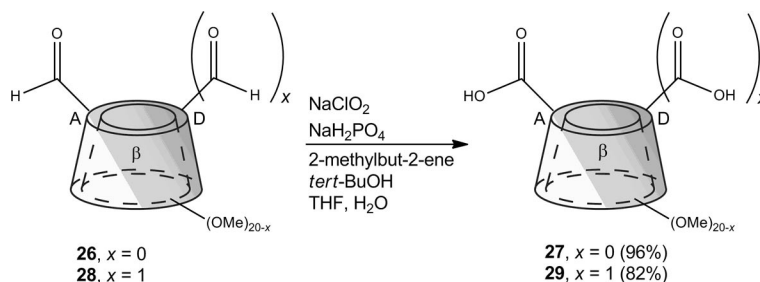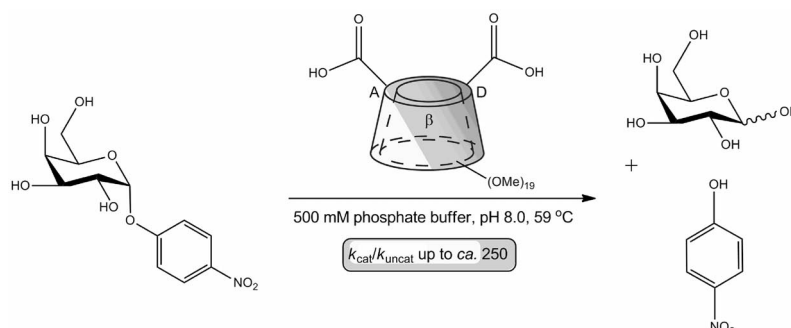

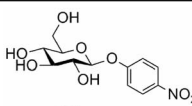Figure 2. Proposed mechanism of carboxylate CD catalysis.

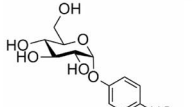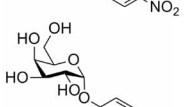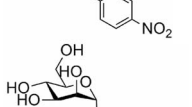Scheme 7. Syntheses of methylated β-CD 6-mono- and $6^A,6^D$-dicarboxylic acids **27** and **29**.

Table 5. Kinetic parameters for the hydrolysis of aryl glycosides in 500 mM phosphate buffer at pH 8.0, 59 °C, catalyzed by permethylated $6^A,6^D$-dicarboxylic acid β-CD (**29**); n.d.: not determined.

Substrate	k_{cat} (10^{-5} s^{-1})	K_m (mM)	k_{cat}/k_{uncat}
	0.17±0.01	n.d.	122±11
	0.11±0.03	n.d.	23±7
	0.32±0.01	3.58±0.24	224±6
	0.12±0.03	n.d.	22±6

expected to function on a very limited number of bound conformers. For the diacids electrostatic interaction with the transition state has been proposed to be responsible for the catalysis and this is expected to be much less strict in terms of conformational demands.

Conclusions

A novel $7^A,7^D$-dicyanohydrin β-cyclodextrin was synthesized and shown to act as an artificial glycosidase. This compound catalyzes the hydrolysis of a range of different aryl glycosides with up to 5500 times rate increase (k_{cat}/k_{uncat}) and in 50 mM phosphate buffer, the catalysis is in all cases superior to previously reported results for $6^A,6^D$-dicyanohydrin CDs. Since these CD glycosidase mimics differ by one carbon atom in the positioning of the catalytic functionality relative to the substrate binding site, this provides proof of substantial flexibility within the catalysis effected by the CD chemzymes. 6^A-Mono- and $6^A,6^D$-dicyanohydrins of both permethylated α- and β-CDs were synthesized, and the monocyanohydrin isomers with the hydroxy group pointing towards the CD cavity, in accordance with the proposed mechanism, catalyzed glycoside hydrolysis with up to 110 times rate enhancement. This catalytic activity is 10-fold smaller than for that of the non-methylated mono-cyanohydrin CD, which suggests that the per-

methylation blocks catalytically important binding modes between CD and substrate. The permethylated $6^A,6^D$-dicarboxylic acid β-CD was synthesized for comparison. The inherent larger talent of this carboxylate CD for overcoming nonpolar permethylation effect makes it possible for this compound to display still 1/4 of the catalytic activity of the nonpermethylated $6^A,6^D$-dicarboxylic acid β-CD. These findings is in accordance with the expected conformational demands of each type of catalyst and afford a deeper understanding of the factors governing glycosidase chemzyme catalysis. Based on these discoveries, novel efforts directed at optimizing the chemzyme design for gaining greater catalysis can be undertaken, which brings about optimism for future discoveries and developments in the field of artificial enzymes.

Experimental Section

General: Solvents were distilled under anhydrous conditions. Evaporation was carried out on a rotary evaporator. All reagents were used as purchased without further purification. For flash column chromatography, silica gel 60 (230–400 mesh) was used as the stationary phase. TLC plates (Merck 60 F_{254}) were visualized by spraying with cerium sulfate (1%) and molybdic acid (1.5%) in H_2SO_4 (10%), and heating until colored spots appeared. NMR experiments were performed on a Varian Mercury 400 instrument,

and a Varian Mercury 300 instrument. Monoisotopic mass spectra (MALDI-TOF MS) were obtained on a Bruker Daltonics mass spectrometer using a 2,5-dihydroxybenzoic acid (DHB) matrix. Spectra were calibrated using a standard peptide calibration kit. Catalysis assays were performed using a Spectronic Genesys 5 spectrophotometer.

Procedure for Determining the Rate of Hydrolysis: Each assay was performed on 1 mL samples prepared from 0.5 mL solutions of the appropriate aryl glycoside at different concentrations mixed with 0.5 mL of phosphate buffer containing either cyclodextrin derivative or nothing as control. Cyclodextrin concentration $[E]_o$ was between 0.12–0.25 mM. Substrate concentration was between 0.6–50 mM. The reactions were followed continuously at 59 °C using UV absorption at 400 nm. The reactions were monitored for 2–6 h. Velocities were determined as the slope of the progress curve of each reaction. Uncatalyzed velocities were obtained directly from the control samples. Catalyzed velocities were calculated by subtracting the uncatalyzed velocity from the velocity of the appropriate cyclodextrin-containing sample. The catalyzed velocities were used to construct a Hanes plot ($[S]/V$ vs. $[S]$) to check that the reaction followed the Michaelis–Menten rate law. K_m and V_{max} were determined from nonlinear regression fitting to the Michaelis–Menten equation using the programme Dataplot.[30] k_{cat} was calculated as $V_{max}/[cyclodextrin]$. k_{uncat} was determined as the slope from a plot of V_{uncat} vs. $[S]$.

$6^A,6^D$-Di-C-aminomethyl-$2^{A–G},3^{A–G},6^{B,C,E–G}$-nonadecakis-$O$-benzyl-$6^A,6^D$-dideoxy-β-cyclodextrin (2): $2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-cyano-$6^A,6^D$-dideoxy-β-cyclodextrin (1) (300 mg, 0.105 mmol) was dissolved in dry CH_2Cl_2 (11 mL) at –78 °C under nitrogen atmosphere. DIBAL-H (0.7 mL, 1.5 M in toluene, 1.047 mmol, 10 equiv.) was added to the solution which was stirred at –78 °C for 1.3 h. The reaction mixture was warmed to 0 °C, aq. HCl (25 mL, 1.0 M) was added, and the organic layer was extracted with Et_2O (4×20 mL). The combined organic extract was washed with sat. aq. $NaHCO_3$ (3×40 mL) and brine (2×30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified consecutively by flash column chromatography (silica, eluent gradient EtOAc/pentane, 1:3→1:0, then: eluent gradient 1% Et_3N in EtOAc/pentane, 1:1→1:0, then: 1% Et_3N in EtOAc/MeOH, 25:1→1:1), which afforded the desired compound (100 mg, 33%) as a colorless solid. $[a]_D^{20} = +53.9$ ($c = 1.0$, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.62$–6.58 (m, 95 H, H_{phenyl}), 5.92–4.23 (m, 44 H), 4.07–3.10 (m, 43 H), 2.80–2.43 (m, 4 H, 7^A-H, 7^D-H) 1.97–1.51 (m, 4 H, 6^A-H, 6^D-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 139.9$–137.8 (C_{ipso}), 129.3–126.2 (CH_{phenyl}), 99.3 (C-1), 99.1 (C-1), 98.9 (C-1), 98.8 (C-1), 98.5 (C-1), 98.3 (C-1), 98.1 (C-1), 81.6, 81.4, 81.0, 81.0, 80.9, 80.9, 80.8, 80.2, 79.4, 79.0, 78.9, 78.7, 78.2, 77.6, 76.6, 76.1, 75.8, 75.2, 74.9, 74.4, 74.0, 73.9, 73.8, 73.7, 73.6, 73.5, 73.4, 73.3, 73.1, 73.1, 72.6, 72.6, 72.0 ppm. 71.9, 69.9, 69.5, 69.4, 69.0. MALDI-TOF-MS: m/z calcd. for $C_{177}H_{190}N_2O_{33}$: 2871.325, found 2871.354.

$2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-dideoxy-$6^A,6^D$-di-$C$-(1,3-dithian-2-yl)-β-cyclodextrin (4): 1,3-Dithian (814 mg, 6.77 mmol, 12 equiv.) was added to a solution of nBuLi in hexane (2.83 mL, 1.6 M, 4.53 mmol, 8 equiv.) at 0 °C under nitrogen atmosphere. The solution was stirred for 60 min at 0 °C, then a solution of $2^{A–G},3^{A–G},6^{B,C,E–G}$-nonadecakis-$O$-benzyl-$6^A,6^D$-diiodo-β-cyclodextrin (3) (1.731 g, 0.564 mmol) in a solution of 10% HMPA in dry THF (3.4 mL) was added and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by addition of water (15 mL) and brine (15 mL) and extracted with EtOAc (5×25 mL). The combined organic layer was washed with brine (4×60 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (eluent gradient, EtOAc/pentane, 1:5→1:3), which afforded the desired product (402 mg, 23%) as a light-yellow foam. $[a]_D^{20} = +44.6$ ($c = 1.0$, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.48$–6.88 (m, 95 H, H_{phenyl}), 5.84–2.77 (m, 87 H), 2.77–2.24 (m, 7 H, $H_{dithiane}$), 2.24–1.53 (m, 6 H, $H_{dithiane}$), 1.02–0.84 (m, 1 H, $H_{dithiane}$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 139.8$–138.2 (C_{ipso}), 128.8–127.1 (CH_{phenyl}), 100.0–97.4 ($7 \times$ C-1), 82.9, 81.2, 81.1, 80.2, 78.9, 75.6, 73.7, 73.6, 73.1, 72.8, 72.0, 71.6, 69.4, 65.5, 30.9 ($C_{dithiane}$), 30.7 ($C_{dithiane}$), 26.3 ($C_{dithiane}$), 26.4 ($C_{dithiane}$) ppm. MALDI-TOF-MS: m/z calcd. for $C_{183}H_{196}O_{33}S_4Na$: 3072.244, found 3072.011.

$2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-dideoxy-$6^A,6^D$-di-$C$-formyl-β-cyclodextrin (5): $2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-dideoxy-$6^A,6^D$-di-$C$-(1,3-dithian-2-yl)-β-cyclodextrin (4) (361 mg, 0.118 mmol) was dissolved in a mixture of water and THF (1:9, 10 mL). Then, a mixture of red HgO (692 mg, 3.20 mmol, 27 equiv.) and $BF_3\cdot Et_2O$ (0.45 mL, 3.55 mmol, 30 equiv.) in THF (5 mL) was added, and the resulting mixture was stirred at room temperature overnight. Reaction progress was checked by MALDI-TOF-MS. The reaction mixture was diluted with water (15 mL) and extracted with EtOAc (5×40 mL). The combined organic extract was washed with brine (2×70 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (eluent gradient, EtOAc/pentane, 1:5→1:3), which afforded the desired product (150 mg, 44%) as a colorless foam. $[a]_D^{20} = +48.5$ ($c = 0.5$, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.45$ (d, $J = 2.7$ Hz, 1 H, CHO), 9.36 (d, $J = 1.8$ Hz, 1 H, CHO), 7.40–6.90 (m, 95 H, H_{phenyl}), 5.43 (d, $J_{1,2} = 3.9$ Hz, 1 H, 1-H), 5.32 (d, $J_{1,2} = 3.6$ Hz, 1 H, 1-H), 5.30–3.29 (m, 77 H), 3.26 (m, 1 H, $5^{A/D}$-H), 3.21 (m, 1 H, $5^{A/D}$-H), 3.15 (m, 1 H, $5^{A/D}$-H), 3.10 (m, 1 H, $5^{A/D}$-H), 2.47 (dd, $J_{5,6} = 9.6$, $J_{6,7} = 2.7$ Hz, 1 H, $6^{A/D}$-H), 2.45–2.37 (m, 2 H, $6^{A/D}$-H), 2.36 (dd, $J_{5,6} = 9.6$, $J_{6,7} = 1.8$ Hz, 1 H, $6^{A/D}$-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 200.4$ (C=O), 200.2 (C=O), 139.5–138.1 (C_{ipso}), 129.6–126.9 (CH_{phenyl}), 99.7 (C-1), 99.4 (C-1), 99.0 (C-1), 98.5 (C-1), 98.4 (C-1), 97.8 (C-1), 97.8 (C-1), 81.4, 80.7, 80.6, 80.3, 79.8, 79.5, 78.9, 78.6, 78.4, 76.5, 75.9, 74.9, 74.7, 73.4, 73.2, 72.8, 72.6, 71.6, 71.2, 69.5, 68.8, 66.5, 46.6 (CH_2-CHO), 46.5 (CH_2-CHO) ppm. MALDI-TOF-MS: m/z calcd. for $C_{177}H_{184}O_{35}Na$: 2892.252, found 2892.286.

$2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-cyano-(hydroxy)methyl-$6^A,6^D$-dideoxy-β-cyclodextrin (6): A mixture of potassium cyanide (483 mg, 7.42 mmol, 142 equiv.) and NH_4Cl (598 mg, 11.18 mmol, 214 equiv.) in water (7.5 mL) at 0 °C were added to a 0 °C solution of $2^{A–G},3^{A–G},6^{B,C,E–G}$-nonadecakis-$O$-benzyl-$6^A,6^D$-dideoxy-$6^A,6^D$-di-$C$-formyl-β-cyclodextrin (5) (150 mg, 5.22×10^{-5} mol) in a mixture of Et_2O and MeOH (1:1, 7.5 mL). The reaction mixture was stirred overnight at room temperature. Reaction progress was checked by MALDI-TOF-MS. The organic solvent was removed in vacuo and the water phase was extracted with CH_2Cl_2 (5×40 mL). The combined organic layer was washed with water (2×40 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (eluent EtOAc/pentane, 1:4), which afforded the desired product as a colorless foam (66 mg, 43%). IR (KBr): $\tilde{v}$ = 3439, 2924, 2854, 2288 (CN), 1626, 1557, 1457, 1385, 1261, 1166, 1040 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.41$–6.90 (m, 95 H, H_{phenyl}), 5.61–3.18 (m, 85 H), 1.82–1.60 (m, 4 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 139.7$–137.6 (C_{ipso}), 128.7–126.6 (CH_{phenyl}), 121.0 (CN), 118.4 (CN), 100.4 (C-1), 100.1 (C-1), 99.5 (C-1), 99.2 (C-1), 98.8 (C-1), 98.4 (C-1), 97.6 (C-1), 82.1, 80.8, 80.5, 80.0, 80.0,

79.1, 77.5, 75.7, 73.9, 73.8, 73.8, 73.7, 73.7, 73.1, 73.0, 7.6, 71.6, 70.3, 69.8, 69.1, 67.6, 60.7, 58.2, 57.9, 38.4 ppm. MALDI-TOF-MS: m/z calcd. for $C_{179}H_{186}N_2O_{35}Na$: 2946.273, found 2946.369 [NMR spectra show the presence of up to 4 enantiomers and diastereomers: $7^A,7^D$ (R,R), (R,S),(S,R), and (S,S), and rotamers of each of these].

Iodo-cyano-triphenylphosphorane: Triphenylphosphane (4.00 g, 15.3 mmol) and iodoacetonitrile (1.44 mL, 19.8 mmol, 1.3 equiv.) were dissolved in toluene (7.0 mL) at room temperature under nitrogen atmosphere and light-free conditions. The reaction mixture was heated to 100 °C and stirred overnight at 100 °C. The resulting precipitated dark crystals were washed with cold toluene (200 mL) and dried under vacuum overnight, this procedure was repeated the next day followed by drying in vacuo overnight, affording the desired product (6.26 g, 96%). IR (KBr): ṽ = 3443, 3053, 3005, 2990, 2772, 2692, 2249 (CN), 1484, 1483, 1435, 1112 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 7.96–7.84 (m, 9 H, H$_{phenyl}$), 7.75–7.70 (m, 6 H, H$_{phenyl}$), 6.00 (d, J_{gem} = 15.2 Hz, 2 H, CH₂) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 136.4, 136.4 ($J_{Cpara,P}$ = 3.0 Hz, CH aromatic, 3 × C$_p$), 134.6, 134.5 ($J_{Cortho/Cpara,P}$ = 10.7 Hz, CH aromatic, 6 × C$_o$/C$_m$), 131.0, 130.9 ($J_{Cortho/Cpara,P}$ = 13.8 Hz, CH aromatic, 6 × C$_o$/C$_m$), 116.4, 115.5 ($J_{Cipso,P}$ = 88.5 Hz, 3 × C$_{ipso}$), 111.8, 111.8 ($J_{CN,P}$ = 9.2 Hz, CN), 19.1, 18.6 ($J_{HCH,P}$ = 54.9 Hz, CH₂) ppm. ³¹P NMR (162 MHz, CDCl₃): δ = 22.5 ppm. HR-MS (ES) m/z calcd. for $C_{20}H_{17}NP$: 302.1099, found 302.1104.

$2^{A–F},3^{A–F},6^{B,C,E,F}$-Hexadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-cyanomethylene-$6^A,6^D$-dideoxy-α-cyclodextrin (8): Iodo-cyano-triphenylphosphorane (763 mg, 1.78 mmol, 5.0 equiv.) was dissolved in freshly distilled THF (14 mL) at room temperature under nitrogen atmosphere. The solution was cooled to –40 °C and nBuLi (1.6 M in hexanes, 1.07 mL, 4.8 equiv.) was added dropwise. The mixture was stirred for 40 min at –40 °C under nitrogen atmosphere. $2^{A–F},3^{A–F},6^{B,C,E,F}$-Hexadecakis-$O$-benzyl-$6^A,6^D$-dioxo-α-cyclodextrin (7) (857 mg, 0.36 mmol) in freshly distilled THF (5 mL) was added to the reaction mixture, which 1 h later was allowed to reach room temperature. Reaction progress monitoring by TLC (silica, eluent EtOAc/pentane, 1:3) showed that the reaction was complete. Diethyl ether (200 mL) was added and the solution was washed with sat. aq. NH₄Cl (2 × 70 mL), water (2 × 120 mL), brine (120 mL), and water (120 mL). The organic layer was dried (MgSO₄), filtered, and concentrated in vacuo. The product was purified by flash column chromatography (eluent EtOAc/pentane, 2:9) affording the desired product (642 mg, 74%) as a colorless solid. $[a]_D$ = +37.8 (c = 0.3, CDCl₃). IR (film): ṽ = 3063, 3029, 2924, 2224 (CN), 1496, 1453, 1354, 1207, 1095, 1039, 1027, 734, 696 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 7.40–6.91 (m, 82 H, 80 H$_{phenyl}$, 6^A-H, 6^D-H), 5.81 [d, $J_{6,CH-CN(trans)}$ = 16.8 Hz, 0.2 H, CH-CN], 5.38 [d, $J_{6,CH-CN(cis)}$ = 12.0 Hz, 0.1 H, CH-CN], 5.17–4.91 (m, 9 H), 4.84–4.65 (m, 9.5 H), 4.52–4.15 (m, 19 H), 4.09–3.88 (m, 12.5 H), 3.86–3.75 (m, 4 H), 3.71–3.60 (m, 4 H), 3.50–3.38 (m, 6 H), 3.27–3.16 (m, 6 H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 151.0 (2× CH=CHCN), 139.5 (2× C$_{ipso}$), 139.3 (2× C$_{ipso}$), 139.2 (2× C$_{ipso}$), 138.5 (2× C$_{ipso}$), 138.4 (2× C$_{ipso}$), 138.2 (2× C$_{ipso}$), 138.0 (4× C$_{ipso}$), 128.7–127.2 (CH$_{aromatic}$), 117.4 (2× CN), 101.4 (2× CHCN), 99.0 (2×C-1), 98.9 (2×C-1), 98.4 (2×C-1), 84.8, 81.2, 81.0, 80.7, 79.8, 79.3, 78.8, 78.7, 78.4, 75.9, 75.9, 75.4, 73.8, 73.5, 73.4, 73.2, 72.1, 71.6, 70.0, 69.1, 68.4 ppm. MALDI-TOF-MS: m/z calcd. for $C_{152}H_{154}N_2O_{28}Na$: 2478.0585, found 2478.329.

$2^{A–F},3^{A–F},6^{B,C,E,F}$-Hexadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-cyano(hydroxy)-methyl-α-cyclodextrin (9): To a solution of $2^{A–F},3^{A–F},6^{B,C,E,F}$-hexadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-cyanomethylene-$6^A,6^D$-dideoxy-α-cyclodextrin (8) (83 mg, 0.034 mmol) in THF/H₂O (4:3, 1.1 mL)

were added NaIO₄ (30.4 mg, 0.142 mmol, 4.2 equiv.) and citric acid (14.2 mg, 0.068 mmol, 2.0 equiv.). The mixture was stirred at 0 °C for 30 min, then OsO₄ (2.5 wt.-% in BuOH, 0.1 mL, 0.008 mmol, 0.23 equiv.) was added dropwise. The reaction was stirred overnight on a slowly melting ice bath. Reaction progress was checked by TLC (silica, eluent EtOAc/pentane, 1:3). The reaction was quenched with 10% aq. NaHSO₄ (28 mL) and stirred for 20 min. The mixture was extracted with EtOAc (3 × 75 mL) and the combined organic layer was washed with sat. aq. Na₂S₂O₃ (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried (MgSO₄), filtered, and concentrated in vacuo. The resulting beige foam underwent flash column chromatography (eluent gradient, EtOAc/pentane, 2:5→EtOAc) to afford the desired product (57 mg, 67%) as a colorless solid. $[a]_D$ = +27.0 (c = 1.1, CDCl₃). IR (film): ṽ = 3415, 3030, 2926, 2860, 2233 (CN), 1496, 1453, 1356, 1207, 1095, 1028, 733, 696 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 7.40–6.78 (m, 80 H, H$_{phenyl}$), 5.83–5.64 (m, 0.5 H), 5.40 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 5.29 (d, $J_{1,2}$ = 4.0 Hz, 0.5 H, 1-H), 5.27–4.51 (m, 20 H), 4.51–4.13 (m, 19.5 H), 4.13–3.76 (m, 18.5 H), 3.76–3.16 (m, 14 H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 139.5–137.3 (C$_{ipso}$), 129.2–126.3 (CH$_{phenyl}$), 119.0 (CN), 101.4 (C-1), 99.9 (C-1), 99.3 (C-1), 99.3 (C-1), 97.8 (C-1), 97.6 (C-1), 82.0, 81.4, 81.1, 80.7, 80.0, 78.8, 76.4, 75.6, 74.4, 74.1, 74.0, 73.8, 73.2, 73.0, 72.9, 72.5, 72.3, 71.5, 71.0, 70.3, 70.0, 68.6, 67.4, 64.7, 63.6, 62.5, 61.5 ppm. MALDI-TOF-MS: m/z calcd. for $C_{152}H_{158}N_2O_{32}Na$: 2546.0695, found 2546.353.

$2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-(E)-cyano-methylene-$6^A,6^D$-dideoxy-β-cyclodextrin (11): Iodo-cyano-triphenylphosphorane (2.52 g, 5.873 mmol, 10 equiv.) in freshly distilled THF (40 mL) was cooled to 0 °C under nitrogen atmosphere. NaH (235 mg, 5.87 mmol, 10 equiv.) was added and the mixture was stirred for 20 min. Next, the mixture was cooled to –40 °C and stirred for another 60 min; then $2^{A–G},3^{A–G},6^{B,C,E–G}$-nonadecakis-$O$-benzyl-$6^A,6^D$-dioxo-β-cyclodextrin (10) (1.67 g, 0.587 mmol) in freshly distilled THF (25 mL) was added. The reaction mixture was stirred for 1.5 h at –40 °C under nitrogen atmosphere and was thereafter allowed to reach room temperature. Reaction progress was monitored by TLC (eluent EtOAc/pentane, 1:3) and MALDI-TOF-MS. After 7 h, EtOAc (170 mL) was added to the reaction mixture which was then washed with sat. aq. NH₄Cl (3 × 80 mL), water (80 mL), brine (2 × 80 mL), and water (80 mL). The organic layer was dried (MgSO₄), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (eluent gradient EtOAc/pentane, 1:5→EtOAc), affording the desired product as a colorless foam (1.16 g, 68%). $[a]_D^{20}$ = +54.0 (c = 1.0, CH₂Cl₂). IR (film): ṽ = 3062, 3030, 2925, 2868, 2225 (CN), 1496, 1453, 1360, 1265, 1207, 1094, 1039, 1027, 735, 698 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ = 7.25–6.94 (m, 95 H, H$_{phenyl}$), 6.86 (dd, $J_{5,6}$ = 4.4, $J_{6,CH-CN}$ = 16.4 Hz, 2 H, 6^A-H, 6^D-H), 5.27 (dd, $J_{5,CH-CN}$ = 1.6, $J_{6,CH-CN}$ = 16.4 Hz, 2 H, CH-CN), 5.20 (s, 2 H), 5.15 (d, J = 1.6 Hz, 1 H, 1-H), 5.13–4.29 (m, 42 H, 1-H, $5^{A,D}$-H, CH_2-Ph), 4.18 (d, J_{gem} = 17.6 Hz, 1 H, CH-Ph), 4.15 (d, J_{gem} = 17.6 Hz, 1 H, CH-Ph), 3.98–3.21 (m, 36 H, 2-H, 3-H, 4-H, $5^{B,C,E–G}$-H, $6^{B,C,E–G}$-H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 151.9 (C-$6^{A/D}$), 151.5 (C-$6^{A/D}$), 139.6–138.1 (C$_{ipso}$), 128.6–127.0 (CH$_{phenyl}$), 117.4 (CN), 117.2 (CN), 101.2, 100.7, 99.5, 99.3, 99.0, 98.7, 98.4, 98.2, 97.9 (2× C-CN, 7× C-1), 81.3, 81.1, 81.1, 81.0, 81.0, 80.8, 80.7, 80.3, 79.9, 79.8, 79.2, 79.1, 79.0, 78.6, 78.5, 78.3, 77.5, 76.3, 76.1, 75.8, 75.6, 75.3, 75.0, 73.9, 73.8, 73.6, 73.6, 73.5, 73.4, 73.3, 73.1, 72.9, 72.8, 72.4, 72.3, 71.7, 71.6, 71.4, 70.0, 69.7, 69.4, 69.2, 69.0, 69.0 ppm. MALDI-TOF-MS: m/z calcd. for $C_{179}H_{182}N_2O_{33}Na$: 2910.2522, found 2910.1317.

$2^{A–G},3^{A–G},6^{B,C,E–G}$-Nonadecakis-$O$-benzyl-$6^A,6^D$-di-$C$-cyano(hydroxy)-methyl-β-cyclodextrin (12): To a solution of $2^{A–G},3^{A–G},6^{B,C,E–G}$-

nonadecakis-*O*-benzyl-6^A,6^D-di-*C*-(*E*)-cyanomethylene-6^A,6^D-dideoxy-β-cyclodextrin (**11**) (385 mg, 0.133 mmol) in THF/H$_2$O (13:2, 3.0 mL) were added NaIO$_4$ (120 mg, 0.56 mmol, 4.2 equiv.) and citric acid (56 mg, 0.266 mmol, 2.0 equiv.). The mixture was stirred for 10 min at 0 °C, then OsO$_4$ (2.5 wt.-% in BuOH, 0.67 mL, 0.053 mmol, 0.4 equiv.) was added dropwise. The reaction was stirred overnight on a slowly melting ice bath. Reaction progress was checked by TLC (silica, eluent EtOAc/pentane, 1:3). Next day more OsO$_4$ (2.5 wt.-% in BuOH, 0.34 mL, 0.026 mmol, 0.2 equiv.) was added and the reaction was left stirring overnight. More OsO$_4$ (2.5 wt.-% in BuOH, 0.34 mL, 0.026 mmol, 0.2 equiv.) was added and the reaction was again left stirring overnight. The reaction was quenched by addition of 10% aq. NaHCO$_3$ (28 mL) and stirred for 30 min. The mixture was extracted with EtOAc (5 × 25 mL) and the combined organic layer was washed with sat. aq. Na$_2$S$_2$O$_3$ (6 × 25 mL) and brine (3 × 25 mL). The organic layer was dried (MgSO$_4$), filtered, and concentrated in vacuo. The resulting colorless solid underwent flash column chromatography (eluent gradient, EtOAc/pentane, 1:3→EtOAc) to afford the desired product (297 mg, 75%) as a colorless solid. [*a*]$_D$ = +22.3 (*c* = 1.0, CHCl$_3$). IR (KBr): ṽ = 3424, 3028, 2924, 2865, 1496, 1453, 1358, 1208, 1095, 1027, 733, 695 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 7.42–6.87 (m, 95 H, H$_{phenyl}$), 5.64–5.00 (m, 3 H), 5.00–4.13 (m, 44 H), 4.13–3.09 (m, 40 H) ppm. ^{13}C NMR (100 MHz, CDCl$_3$): δ = 138.8–135.7 (C$_{ipso}$), 128.8–125.4 (CH$_{phenyl}$), 118.1 (CN), 118.0 (CN), 99.0 (C-1), 98.9 (C-1), 98.2 (C-1), 97.2 (C-1), 96.2 (C-1), 96.1 (C-1), 96.0 (C-1), 80.5, 80.4, 79.8, 79.6, 79.5, 79.4, 79.0, 78.4, 78.0, 77.7, 77.0, 76.2, 75.1, 74.6, 73.1, 72.8, 72.4, 72.3, 72.1, 71.8, 71.6, 71.5, 71.4, 71.2, 70.7, 70.5, 70.2, 69.9, 69.5, 69.1, 68.3, 68.1, 68.0, 67.5, 65.8, 62.7, 61.8, 61.6, 61.5, 61.2, 61.1, 60.9 ppm. MALDI-TOF-MS: *m/z* calcd. for C$_{179}$H$_{186}$N$_2$O$_{37}$Na: 2978.2632, found 2978.3688.

6^A,6^D-Di-*C*-cyano(hydroxy)methyl-β-cyclodextrin (13):

2^{A-G},3^{A-G},6$^{B,C,E-G}$-Nonadecakis-*O*-benzyl-6^A,6^D-di-*C*-cyano(hydroxy)methyl-β-cyclodextrin (**12**) (216 mg, 0.073 mmol) was dissolved in EtOAc/MeOH (1:1, 15 mL). Pd(OH)$_2$/C 20% (200 mg) and TFA (cat.) were added and the reaction mixture was stirred at room temperature under hydrogen atmosphere until completion. Filtration through a bed of celite and removal of the solvent afforded the desired product (94 mg, 100%) as a colorless powder. [*a*]$_D$ = +73.2 (*c* = 0.5, D$_2$O). IR (KBr): ṽ = 3395, 2933, 2233 (CN), 1678, 1431, 1367, 1203, 1034 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 5.16–4.92 (m, 7 H, 1-H), 4.27–3.43 (m, 42 H) ppm. ^{13}C NMR (75 MHz, D$_2$O): δ = 117.3 (CN), 102.3–101.9 (7 × C-1), 81.4, 81.3, 81.2, 81.1, 81.0, 73.4, 73.3, 72.2, 72.1, 72.0, 71.9, 71.9, 71.9, 71.9, 71.9, 71.2, 71.2, 71.2, 71.2, 71.2, 69.5, 69.5, 69.5, 69.5, 69.5, 62.7, 62.7, 60.5, 60.5, 60.5, 60.5, 60.3, 60.2, 58.2, 58.2, 58.2, 58.2 ppm. MALDI-TOF-MS: *m/z* calcd. for C$_{46}$H$_{72}$N$_2$O$_{37}$Na: 1267.3711, found 1266.7610.

6^A,6^D-Di-*O*-acyl-2^{A-G},3^{A-G},6$^{B,C,E-G}$-nonadecakis-*O*-benzyl-6^A,6^D-di-*C*-cyano-β-cyclodextrin (15):

2^{A-G},3^{A-G},6$^{B,C,E-G}$-Nonadecakis-*O*-benzyl-6^A,6^D-di-*C*-cyano-β-cyclodextrin (**14**) (939 mg, 324 mmol) was dissolved in dry CH$_2$Cl$_2$ (3.0 mL) under nitrogen atmosphere and cooled to 0 °C. Ac$_2$O (300 µL, 3.24 mmol, 10 equiv.) and pyridine (235 µL, 2.917 mmol, 9 equiv.) were added to the solution which was stirred at room temperature for 6 h. Reaction progress was monitored by TLC (eluent, EtOAc/pentane, 1:3). The reaction mixture was washed 3 times with HCl (3% in water), and once with brine, then dried (MgSO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (eluent gradient, EtOAc/pentane, 1:5→1:3), affording the desired product (887 mg, 92%) as a colorless solid. [*a*]$_D^{20}$ = +48.8 (*c* = 2.0, CH$_2$Cl$_2$), IR (film, CH$_2$Cl$_2$): ṽ = 3054, 2987, 1605, 1551, 1496, 1454, 1422, 1360, 1264, 1216, 1095, 1040, 896, 804, 738 cm^{-1}. ^{1}H NMR

(300 MHz, CDCl$_3$): δ = 7.60–6.80 (m, 95 H, H$_{phenyl}$), 5.63–5.42 (m, 2 H, 1-H), 5.41–3.23 (m, 83 H), 2.02 [s, 3 H, C(O)CH$_3$], 1.79 [s, 3 H, C(O)CH$_3$] ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ(ppm) 169.6 (C=O), 169.2 (C=O), 140.0–137.9 (C$_{ipso}$), 128.8–126.7 (CH$_{phenyl}$), 116.8 (CN), 116.7 (CN), 101.0–97.7 (7 × C-1), 82.2–69.0 (C-2, C-3, C-4, C-5, C-6 $^{B,C,E-G}$, CH$_2$-Ph), 53.8 (C-6^A, C-6^D), 20.6 (*CH$_3$*-C=O), 20.3 (*CH$_3$*-C=O). MALDI-TOF-MS: *m/z* calcd. for C$_{181}$H$_{186}$N$_2$O$_{37}$Na: 3002.263, found 3000.741.

2^{A-G},3^{A-G},6^{B-G}-Eiocosakis-*O*-benzyl-6^A-*C*-cyano-6^A-*O*-trimethylsilyl-β-cyclodextrin (17):

2^{A-G},3^{A-G},6^{B-G}-Eiocosakis-*O*-benzyl-6^A-oxo-β-cyclodextrin (**16**) (87 mg, 0.030 mmol) was dissolved in dry CH$_2$Cl$_2$ (2.0 mL) under nitrogen atmosphere. Et$_3$N (0.8 µL, 0.006 mmol, 0.2 equiv.) and TMSCN (74 µL, 0.593 mmol, 20 equiv.) were added and the reaction was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue was purified by flash column chromatography (eluent EtOAc/pentane, 2:7), affording the desired product as a colorless solid (74 mg, 82%). [*a*]$_D^{20}$ = +31.8 (*c* = 1.0, CHCl$_3$). IR (KBr): ṽ = 3439, 2921, 2855, 1632, 1454, 1094, 1036 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 7.34–6.97 (m, 100 H, H$_{phenyl}$), 5.57 (d, *J* = 1.5 Hz, 0.5 H, 1-H), 5.39 (d, *J* = 3.6 Hz, 0.5 H, 1-H), 5.34 (d, *J* = 3.6 Hz, 0.5 H, 1-H), 5.32 (d, *J* = 3.9 Hz, 0.5 H, 1-H), 5.27 (d, *J* = 3.9 Hz, 0.5 H, 1-H), 5.24–3.36 (m, 85.5 H), 0.17 (s, 4.5 H, TMS), 0.05 (s, 4.5 H, TMS) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 139.6–137.8 (C$_{ipso}$), 128.9–126.7 (CH$_{phenyl}$), 119.1 (CN), 117.4 (CN), 99.4 (C-1), 99.1 (C-1), 99.1 (C-1), 98.9 (C-1), 98.9 (C-1), 98.8 (C-1), 98.5 (C-1), 81.1, 81.0, 80.9, 80.6, 80.3, 80.0, 79.3, 79.2, 79.1, 78.8, 77.3, 76.0, 75.4, 73.6, 73.4, 73.4, 73.3, 73.3, 73.2, 72.8, 72.7, 72.6, 71.9, 71.7, 71.5, 69.5, 69.4, 69.3, 63.1, 61.4, 0.5 (TMS), 0.0 (TMS) ppm. MALDI-TOF-MS: *m/z* calcd. for C$_{186}$H$_{197}$NO$_{35}$SiNa: 3055.333, found 3056.765.

6^A-*C*-Cyano-2^{A-F},3^{A-F},6^{B-F}-heptadecakis-*O*-methyl-α-cyclodextrin (19):

HCl (2.0 M, 9.9 mL) was added to a mixture of 2^{A-F},3^{A-F},6^{B-F}-heptadecakis-*O*-methyl-6^A-oxo-α-cyclodextrin (**18**) (243 mg, 0.201 mmol) and potassium cyanide (1.308 g, 20.09 mmol, 100 equiv.) at 5 °C. The reaction mixture was stirred overnight at room temperature. Reaction progress was checked by TLC (eluent: 6% MeOH in CH$_2$Cl$_2$). The aqueous layer was extracted with CH$_2$Cl$_2$, dried (MgSO$_4$), filtered, and concentrated in vacuo along with silica gel. The residue was purified by flash column chromatography (eluent gradient, CH$_2$Cl$_2$/MeOH, 0:1→97:3), affording the desired product (170 mg, 69%) as a colorless solid. There were nearly equal amounts of the cyanohydrin (*S*)- and (*R*)-isomer. (*R*)-isomer (most apolar): [*a*]$_D^{20}$ = +147.1 (*c* = 1.0, MeOH). IR (KBr): ṽ = 3435, 2925, 2855, 1630, 1490, 1083 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.38 (d, *J*$_{1,2}$ = 2.1 Hz, 1 H, 1-H), 5.23 (d, *J*$_{1,2}$ = 3.3 Hz, 1 H, 1-H), 5.13–5.06 (m, 4 H, 1-H), 4.16–4.02 (m, 2 H), 3.96–3.74 (m, 11 H), 3.74–3.66 (m, 20 H), 3.66–3.52 (m, 29 H), 3.48–3.45 (m, 6 H), 3.45–3.40 (m, 10 H), 3.39–3.35 (m, 2 H), 3.27–3.14 (m, 6 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 119.2 (CN), 100.9 (C-1), 100.4 (2 × C-1), 100.3 (2 × C-1), 100.2 (C-1), 84.3, 83.4, 83.4, 83.3, 83.2, 83.1, 83.1, 83.1, 83.0, 83.0, 82.9, 82.8, 82.8, 82.6, 74.2, 72.9, 72.9, 72.8, 72.7, 72.7, 72.4, 72.4, 72.3, 63.5, 62.1, 62.1, 62.1, 59.4, 59.3, 59.3, 59.3, 59.2, 58.6, 58.6, 58.6, 58.5, 58.5 ppm. MALDI-TOF-MS: *m/z* calcd. for C$_{54}$H$_{93}$NO$_{30}$Na: 1258.568, found 1258.711.

(*S*)-isomer (most polar): [*a*]$_D^{20}$ = +125.7 (*c* = 1.0, MeOH). IR (KBr): ṽ = 3436, 2929, 2859, 2272, 1636, 1420, 1384, 1161, 1056 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.51 (br. s, 1 H, 1-H), 5.19 (d, *J*$_{1,2}$ = 3.0 Hz, 1 H, 1-H), 5.10 (d, *J*$_{1,2}$ = 3.3 Hz, 1 H, 1-H), 5.09–5.05 (m, 3 H, 1-H), 4.26 (dd, *J* = 3.3, *J* = 11.7 Hz, 1 H), 4.04 (dd, *J* = 1.2, *J* = 9.3 Hz, 1 H), 3.96–3.82 (m, 8 H), 3.79–3.60 (m, 25 H),

3.60–3.49 (m, 28 H), 3.45–3.43 (s, 6 H), 3.43–3.40 (m, 4 H), 3.40–3.37 (m, 7 H), 3.21–3.11 (m, 6 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 120.8 (CN), 100.4 (2 × C-1), 100.0 (2 × C-1), 99.9 (C-1), 99.8 (C-1), 83.4, 83.0, 83.0, 83.0, 83.0, 82.9, 82.9, 82.8, 82.7, 82.6, 82.4, 82.1, 81.9, 81.5, 74.0, 73.3, 72.9, 72.8, 72.6, 72.5, 72.2, 72.1, 72.0, 71.8, 62.0, 61.9, 61.9, 61.7, 61.5, 61.0, 59.3, 59.3, 59.1, 59.1, 58.9, 58.7, 58.5, 58.3, 58.1, 58.0 ppm. MALDI-TOF-MS: m/z calcd. for C$_{54}$H$_{93}$NO$_{30}$Na: 1258.568, found 1258.666.

6^{A},6^{D}-Di-C-cyano-2$^{A–F}$,3$^{A–F}$,6^{B,C,E,F}-hexadecakis-O-methyl-α-cyclodextrin (21): HCl (2.0 M, 0.8 mL) was added to a mixture of 6^{A,D}-dioxo-2$^{A–F}$,3$^{A–F}$,6$^{B–C,E–F}$-hexadecakis-O-methyl-α-cyclodextrin (**20**) (14.3 mg, 0.012 mmol) and potassium cyanide (117 mg, 1.798 mmol, 150 equiv.) in water (0.2 mL) at 5 °C. The reaction mixture was stirred overnight at room temperature. Reaction progress was checked by TLC (eluent: 6% MeOH in CH$_2$Cl$_2$). The aqueous layer was extracted with CH$_2$Cl$_2$ (5 × 10 mL), dried (MgSO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (eluent gradient, CH$_2$Cl$_2$/MeOH, 0:1 → 97:3), affording the desired product (14.3 mg, 96%) as a colorless solid. All three 6^{A},6^{D} stereogenic position isomers were present.

(*R,R*)-isomer (most apolar): [a]$_D^{20}$ = +49.0 (*c* = 0.17, MeOH). IR (KBr): ṽ = 3436, 2928, 2858, 1636, 1629, 1487, 1364, 1163, 1096, 1041 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.34 (d, $J_{1,2}$ = 2.1 Hz, 1 H, 1-H), 5.17 (d, $J_{1,2}$ = 2.7 Hz, 1 H, 1-H), 5.09–4.98 (m, 4 H, 1-H), 4.03–3.67 (m, 13 H), 3.67–3.58 (m, 18 H), 3.58–3.44 (m, 27 H), 3.44–3.35 (m, 15 H), 3.23–3.05 (m, 9 H) ppm. MALDI-TOF-MS: m/z calcd. for C$_{54}$H$_{90}$N$_2$O$_{30}$Na: 1269.548, found 1269.851.

(*R,S*) = (*S,R*)-isomer [and small amounts of (*S,S*)-isomer]: [a]$_D^{20}$ = +59.6 (*c* = 0.5, MeOH). IR (KBr): ṽ = 3436, 2918, 2851, 1630, 1490, 1385, 1262, 1095, 1029 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.56 (d, $J_{1,2}$ = 1.5 Hz, 0.5 H, 1-H), 5.41 (d, $J_{1,2}$ = 1.8 Hz, 0.5 H, 1-H), 5.23–5.17 (m, 2 H, 1-H), 5.11 (d, $J_{1,2}$ = 3.6 Hz, 0.5 H, 1-H), 5.09–5.03 (m, 2.5 H, 1-H), 4.25–3.70 (m, 9 H), 3.70–3.59 (m, 24 H), 3.59–3.48 (m, 26 H), 3.48–3.37 (m, 15 H), 3.27–3.12 (m, 8 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 100.7 (C-1), 100.7 (C-1), 100.1 (C-1), 84.6, 83.2, 83.1, 83.0, 82.9, 82.8, 82.7, 81.9, 74.2, 73.6, 73.0, 72.9, 72.3, 72.2, 62.2, 62.2, 61.3, 59.5, 59.5, 59.2, 58.7 ppm. MALDI-TOF-MS: m/z calcd. for C$_{54}$H$_{90}$N$_2$O$_{30}$Na: 1269.548, found 1269.528.

(*S,S*)-isomer (most polar): [a]$_D^{20}$ = +55.2 (*c* = 0.5, MeOH). IR (KBr): ṽ = 3435, 2962, 2927, 2858, 1629, 1490, 1262, 1083, 1028 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.62 (br. s, 1 H, 1-H), 5.23–5.14 (m, 2 H, 1-H), 5.10 (d, $J_{1,2}$ = 3.9 Hz, 1 H, 1-H), 5.08–5.02 (m, 2 H, 1-H), 4.34 (d, J = 11.1 Hz, 0.5 H), 4.09–3.80 (m, 5.5 H), 3.80–3.60 (m, 25 H), 3.60–3.47 (m, 27 H), 3.47–3.37 (m, 15 H), 3.25–3.09 (m, 9 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 121.1 (2 × CN), 101.0 (2 × C-1), 100.4 (2 × C-1), 99.7 (2 × C-1), 83.7, 83.3, 83.1, 83.1, 82.7, 82.6, 82.5, 82.2, 82.1, 74.4, 73.8, 72.4, 72.4, 71.7, 62.4, 62.3, 61.5, 61.3, 59.9, 59.8, 59.5, 58.7, 57.7 ppm. MALDI-TOF-MS: m/z calcd. for C$_{54}$H$_{90}$N$_2$O$_{30}$Na: 1269.548, found 1269.348.

6^{A}-C-Cyano-2$^{A–G}$,3$^{A–G}$,6$^{B–G}$-eicosakis-O-methyl-β-cyclodextrin (23): HCl (2.0 M, 2.0 mL) was added to a mixture of 2$^{A–G}$,3$^{A–G}$,6$^{B–G}$-eicosakis-O-methyl-6^{A}-oxo-β-cyclodextrin (**22**) (53 mg, 0.0375 mmol) and potassium cyanide (244 mg, 3.75 mmol, 100 equiv.) at 5 °C. The reaction mixture was stirred overnight at room temperature. Reaction progress was checked by TLC (eluent: 6% MeOH in CH$_2$Cl$_2$) and MALDI-TOF-MS. The aqueous layer was extracted with CH$_2$Cl$_2$ (5 × 5 mL), dried (MgSO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash column

chromatography (eluent gradient, CH$_2$Cl$_2$/MeOH, 0:1 → 24:1), affording the desired product (43.0 mg, 80%) as a colorless solid. TLC indicated that there were about equal amounts of the cyanohydrin (*S*)- and (*R*)-isomers; the bulk purified product was racemic, but a few fractions containing only single (*S*)- or (*R*)-isomer were successfully isolated.

(*R*)-isomer (most apolar): [a]$_D^{20}$ = +141.0 (*c* = 0.2, MeOH). IR (KBr): ṽ = 3436, 3030, 2928, 1724, 1630, 1604, 1496, 1454, 1364, 1274, 1207, 1094, 1041 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.24 (d, $J_{1,2}$ = 3.3 Hz, 1 H, 1-H), 5.20 (d, $J_{1,2}$ = 2.4 Hz, 1 H, 1-H), 5.18–5.09 (m, 5 H, 1-H), 4.44–3.66 (m, 11 H), 3.66–3.60 (m, 21 H, OMe), 3.60–3.52 (m, 9 H), 3.52–3.47 (m, 21 H, OMe), 3.44–3.34 (m, 16 H), 3.33–3.27 (m, 15 H), 3.23 (dd, $J_{1,2}$ = 3.3, $J_{2,3}$ = 9.0 Hz, 2 H, 2-H), 3.19–3.11 (m, 6 H) ppm. MALDI-TOF-MS: m/z calcd. for C$_{63}$H$_{109}$NO$_{35}$Na: 1462.668, found 1462.159.

(*S*)-isomer (most polar): [a]$_D^{20}$ = +127.3 (*c* = 0.2, MeOH). IR (KBr): ṽ = 3435, 2928, 1618, 1490, 1083, 1039 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.49–5.46 (m, 1 H, 1-H), 5.25 (d, $J_{1,2}$ = 3.3 Hz, 1 H, 1-H), 5.19 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.17–5.12 (m, 3 H, 1-H), 5.10 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 3.95–3.66 (m, 9 H), 3.66–3.61 (m, 21 H, OMe), 3.61–3.53 (m, 8 H), 3.53–3.45 (m, 21 H, OMe), 3.43–3.33 (m, 20 H), 3.33–3.27 (m, 14 H), 3.20–3.11 (m, 8 H) ppm. MALDI-TOF-MS: m/z calcd. for C$_{63}$H$_{109}$NO$_{35}$Na: 1462.668, found 1462.358.

(*R,S*) racemic mixture: ^{13}C NMR (75 MHz, CD$_3$OD): δ = 120.9 (CN), 119.2 (CN), 100.4 (C-1), 99.9–99.3 (C-1), 83.6, 83.5, 83.5, 83.5, 83.4, 83.2, 83.2, 83.1, 83.1, 82.9, 82.9, 82.2, 81.4, 81.1, 81.0, 80.8, 80.8, 80.6, 80.6, 80.5, 80.4, 74.1, 74.1, 73.4, 72.8, 72.8, 72.7, 72.7, 72.4, 72.4, 72.3, 72.3, 72.0, 63.3, 62.2, 62.0, 61.9, 61.9, 61.8, 61.8, 61.7, 61.2, 59.6, 59.6, 59.4, 59.4, 59.3, 59.2, 59.1, 59.1, 59.0, 58.9, 58.6 ppm. MALDI-TOF-MS: m/z calcd. for C$_{63}$H$_{109}$NO$_{35}$Na: 1462.668, found 1462.246.

6^{A},6^{D}-Di-C-cyano-2$^{A–G}$,3$^{A–G}$,6$^{B,C,E–G}$-nonadecakis-O-methyl-β-cyclodextrin (25): HCl (1.0 M, 2.3 mL) was added to a mixture of 6^{A},6^{D}-dioxo-2$^{A–G}$,3$^{A–G}$,6$^{B–C,E–G}$-nonadecakis-O-methyl-β-cyclodextrin (**24**) (21 mg, 0.015 mmol) and potassium cyanide (147 mg, 2.255 mmol, 150 equiv.) at 5 °C. The reaction mixture was stirred overnight at room temperature. Reaction progress was checked by TLC (eluent: 6% MeOH in CH$_2$Cl$_2$). The aqueous layer was extracted with CH$_2$Cl$_2$, dried (MgSO$_4$), filtered, and concentrated in vacuo along with silica gel. The residue was purified by flash column chromatography (eluent gradient, CH$_2$Cl$_2$/MeOH, 0:1 → 24:1), affording the desired product (18.5 mg, 85%) as a colorless solid. All 4 possible 6^{A},6^{D}-disubstituted cyanohydrin CD isomers were present. Whilst the (*S,S*)-isomer was plentiful and the major isomer, only trace amounts of the (*R,R*)-isomer were detected.

(*R,S*)- and (*S,R*)-isomer mixture: IR (KBr): ṽ = 3400, 2956, 1508, 1491, 1420, 1385, 1162, 1142, 1108, 1035 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.55–5.53 (m, 0.5 H, 1-H), 5.51–5.49 (m, 0.5 H, 1-H), 5.29–5.04 (m, 6 H, 1-H), 4.20–3.70 (m, 11 H), 3.69–3.59 (m, 26 H), 3.59–3.44 (m, 34 H), 3.44–3.34 (m, 18 H), 3.26–3.12 (m, 8 H) ppm. MALDI-TOF-MS: m/z calcd. for C$_{63}$H$_{106}$N$_2$O$_{35}$Na: 1473.647, found 1473.4630.

(*S,S*)-isomer (most polar): [a]$_D^{20}$ = +138.0 (*c* = 1.0, MeOH). IR (KBr): ṽ = 3434, 2927, 2855, 1654, 1636, 1630, 1384, 1033 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.58 (br. s, 1 H, 1-H), 5.30 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.28 (d, $J_{1,2}$ = 2.7 Hz, 1 H, 1-H), 5.25 (d, $J_{1,2}$ = 4.8 Hz, 1 H, 1-H), 5.15 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.10 (d, $J_{1,2}$ = 3.3 Hz, 2 H, 1-H), 4.43–4.21 (m, 2 H), 3.99–3.73 (m, 14 H), 3.73–3.61 (m, 26 H), 3.61–3.48 (m, 32 H), 3.47–3.37 (m, 16 H),

3.25–3.14 (m, 7 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 120.9 (2 × CN), 100.1 (C-1), 100.0 (C-1), 99.9 (3 × C-1), 99.5 (C-1), 99.3 (C-1), 83.7, 83.6, 83.5, 83.5, 83.5, 83.4, 83.3, 83.2, 82.8, 82.8, 82.8, 81.6, 81.4, 80.9, 80.8, 80.2, 79.8, 74.2, 74.2, 73.6, 73.4, 72.9, 72.7, 72.5, 72.5, 72.4, 72.4, 72.3, 72.3, 72.0, 71.7, 62.3, 62.3, 62.0, 62.0, 61.9, 61.6, 61.5, 61.2, 61.2, 60.1, 59.9, 59.7, 59.7, 59.6, 59.6, 59.6, 59.3, 59.2, 59.1, 59.1, 59.0, 58.4, 58.3 ppm. MALDI-TOF-MS: m/z calcd. for C$_{63}$H$_{106}$N$_2$O$_{35}$Na: 1473.647, found 1473.862.

2$^{A–G}$,3$^{A–G}$,6$^{B–G}$-Eiocosakis-O-methyl-β-cyclodextrin-6^A-carboxylic Acid (27): 2$^{A–G}$,3$^{A–G}$,6$^{B–G}$-eiocosakis-O-methyl-6^A-oxo-β-cyclodextrin (**22**) (31 mg, 0.0219 mmol) was dissolved in a mixture of tBuOH (0.85 mL) in THF (0.7 mL). To this were added 2-methyl-2-butene (98 mg, 1.40 mmol, 64 equiv.), and a solution of NaClO$_2$ (35 mg, 80%, 0.307 mmol, 14 equiv.) and NaH$_2$PO$_4$·H$_2$O (32 mg, 0.208 mmol, 9.5 equiv.) in water (0.17 mL). The mixture was stirred overnight at room temperature. Reaction progress was checked by TLC (eluent, 98:9:1 CH$_2$Cl$_2$/MeOH/HCOOH). Upon completion, HCl$_{(aq.)}$ (1.0 M, a few drops), and EtOAc and water were added. The layers were separated and the aqueous layer was extracted with CH$_2$Cl$_2$, the combined organic layer was dried (MgSO$_4$), filtered, and concentrated in vacuo along with silica. The residue was purified by flash column chromatography (eluent gradient, CH$_2$Cl$_2$/MeOH/TFA, 199:0:1 → 193:6:1), affording the desired product (30.0 mg, 96%) as a colorless solid. [a]$_D^{20}$ = +109.4 (c = 1.0, MeOH). IR (KBr): $\tilde{\nu}$ = 3435, 2927, 2860, 1636, 1629, 1496, 1455, 1385, 1364, 1208, 1096, 1041, 1028 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 5.13 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.12 (d, $J_{1,2}$ = 2.7 Hz, 1 H, 1-H), 5.07 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 5.06–4.99 (m, 4 H, 1-H), 4.21 (br. d, J = 8.1 Hz, 1 H), 3.90–3.56 (m, 14 H), 3.56–3.45 (m, 28 H), 3.45–3.33 (m, 28 H), 3.29–3.22 (m, 17 H), 3.22–3.12 (m, 6 H), 3.07–2.98 (m, 6 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 171.9 (C=O), 100.1 (C-1), 99.8 (C-1), 99.6 (C-1), 99.5 (C-1), 99.1 (C-1), 99.0 (2 × C-1), 83.7, 83.4, 83.4, 83.4, 83.4, 83.2, 83.2, 83.1, 83.1, 82.7, 81.7, 81.5, 81.2, 80.8, 80.5, 79.7, 79.0, 73.5, 72.8, 72.7, 72.7, 72.3, 72.3, 72.0, 72.0, 71.8, 61.9, 61.9, 61.8, 61.8, 61.8, 61.8, 61.8, 61.6, 61.6, 61.2, 59.8, 59.8, 59.3, 59.3, 59.3, 59.3, 59.2, 59.2, 59.1, 59.1, 58.9, 58.9, 58.9, 58.9, 58.8, 58.8 ppm. MALDI-TOF-MS: m/z calcd. for C$_{62}$H$_{108}$O$_{36}$Na: 1451.652, found 1452.225.

2$^{A–G}$,3$^{A–G}$,6$^{B–C,E–G}$-Nonadecakis-O-methyl-β-cyclodextrin-6^A,6^D-dicarboxylic Acid (29): 6^A,6^D-Dioxo-2$^{A–G}$,3$^{A–G}$,6$^{B–C,E–G}$-nonadecakis-O-methyl-β-cyclodextrin (**24**) (24.0 mg, 0.017 mmol) was dissolved in a mixture of 2-methylbut-2-ene in THF (1.1 mL, 2.0 M in THF) and tBuOH (1.32 mL) at room temperature. To the mixture was then added a solution of NaClO$_2$ (53 mg, 80%, 0.467 mmol, 27.2 equiv.) and NaH$_2$PO$_4$ monohydrate (44 mg, 0.319 mmol, 18.6 equiv.) in H$_2$O (0.26 mL). The reaction mixture was stirred 15 h at room temperature. The reaction was quenched by addition of aq. HCl (1.0 M, 7.0 mL), the organic layer was saved and the aqueous layer was extracted with EtOAc. The organic phase was dried (MgSO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography [silica gel, eluent CH$_2$Cl$_2$/MeOH (93:7) containing 1% HCOOH], affording the product as a colourless solid (20.0 mg, 0.014 mmol, 82% yield). [a]$_D^{20}$ = +129.0 (c = 1.0, CHCl$_3$). IR (KBr): $\tilde{\nu}$ = 3436, 2932, 2834, 1744, 1630, 1458, 1368, 1142, 1107, 1039 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.43 (d, J = 3.9 Hz, 1 H, 1-H), 5.28 (d, J = 3.6 Hz, 1 H, 1-H), 5.16 (d, J = 3.3 Hz, 1 H, 1-H), 5.08 (d, J = 3.6 Hz, 1 H, 1-H), 5.07 (d, J = 3.6 Hz, 1 H, 1-H), 4.99 (d, J = 3.3 Hz, 1 H, 1-H), 4.97 (d, J = 3.6 Hz, 1 H, 1-H), 4.10–3.34 (m, 88 H), 3.30 (dd, $J_{1,2}$ = 3.0, $J_{2,3}$ = 9.0 Hz, 2 H, 2-H), 3.21–3.13 (m, 5 H, 2-H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 171.3 (COOH),

171.1 (COOH), 99.8 (C-1), 99.7 (C-1), 99.7 (C-1), 98.1 (C-1), 97.6 (C-1), 97.1 (C-1), 97.1 (C-1), 82.9, 82.7, 82.3, 82.2, 82.1, 82.0, 81.9, 81.8, 81.4, 81.2, 80.2, 77.4, 77.1, 76.7, 76.0, 73.0, 72.4, 71.7, 71.4, 71.1, 71.0, 70.9, 70.7, 70.6, 62.1, 62.0, 61.9, 61.7, 60.9, 60.6, 59.8, 59.4, 59.3, 59.2, 58.8, 58.4 ppm. MALDI-TOF-MS: m/z calcd. for C$_{61}$H$_{104}$O$_{37}$Na: 1451.615, found 1450.761.

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