In Situ Generation and Screening of a Dynamic Combinatorial Carbohydrate Library against Concanavalin A

Olof Ramström and Jean-Marie Lehn*[a]

Dynamic combinatorial chemistry (DCC) is a recently introduced approach that is based on the generation of combinatorial libraries by reversible interconversion of the library constituents. In this study, the implementation of such libraries on carbohydrate – lectin interactions was examined. The dynamic carbohydrate libraries were generated from a small set (four or six compounds) of initial carbohydrate dimers through mild disulfide interchange, and selection was performed under two conditions defining either adaptive or pre-equilibrated libraries. Upon initiation, libraries were formed that contained comparable amounts of 10 or 21 individual dimeric species, dynamically interchanging during the scrambling process. They were probed with respect to binding to the plant lectin concanavalin A, either present during library generation or added after equilibration. The libraries could be generated easily both in the presence and absence of the receptor, and a bis-mannose structure was preferentially bound and selected from the mixture. Scrambling of the library in the presence of the receptor resulted in slightly higher yields than when the receptor was added after scrambling, indicating that the receptor to some extent acts as a thermodynamic trap during library generation. The present results illustrate the extention of the DCC approach to carbohydrate recognition groups, the generation of isoenergetic dynamic libraries, and the implementation of either adaptive or pre-equilibrated procedures.

KEYWORDS:

carbohydrates · combinatorial chemistry · disulfide interchange · lectins

Introduction

Usual approaches to combinatorial chemistry are based upon sequential and irreversible syntheses, be they performed individually in parallel, or concertedly in the same compartment, and all constituents of the library are more or less robust molecules. While this methodology offers satisfactory control, its flexibility in library generation is inherently limited, inasmuch as all structures have to be designed distinctly and are produced separately. If, however, dynamic features can be introduced in the generation process, a new dimension of the combinatorial procedure can be envisaged. In this case, the library maintains the flexibility to self-adjust to the chosen target macromolecule at a given time in a certain environment, and by virtue of reversible molecular and supramolecular interchange processes, it can adapt to the system constraints, in particular receptor-induced molecular-recognition-driven selection events.

Dynamic combinatorial chemistry (DCC) is a recently developed approach that gives access to such self-adjusting virtual combinatorial libraries (VCLs) and addresses the issues mentioned above (for reviews see refs. [1 – 3]). The basic features of the DCC/VCL concept have been discussed recently.[3] It is based on dynamic combinatorial libraries (DCLs) consisting of rapidly interchanging constituents, each formed or broken down in situ through a variety of reversible connection processes involving noncovalent interactions, (e.g., metal ion coordination,[4] π stacking,[5] hydrogen bonding,[6] or charge – charge interactions), or reversible covalent reactions.[7–11] The latter are especially attractive, since bond formation and cleavage may occur under particular conditions and be inhibited under others. Dynamic libraries can also be of configurational or conformational character, for example in *cis*,*trans* isomerisation, in which the difference in configuration can be used in the selection process.[12, 13]

Carbohydrate recognition plays an important role in many biological processes, such as cell – cell interactions, cell communication, etc.[14–16] In addition, a multitude of enzymes are involved in various carbohydrate-mediated processes associated with, for example, defence, cell proliferation, and cell death, as well as in general carbohydrate metabolism. Carbohydrate groups are therefore highly attractive tools for generating mimics and analogues of such recognition processes, and many attempts have been made to evaluate the possibility of designing ligands, based on naturally occurring carbohydrates, for direct or indirect inhibition of carbohydrate-recognising enzymes or as potential agonists/antagonists of carbohydrate receptors.[17]

Lectins constitute a large class of carbohydrate-binding proteins of which concanavalin A (Con A) is the most studied.[18] This plant lectin is specific for a branched trimannoside core unit,

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located in N-glycosidic carbohydrate – peptide linkages of glycoproteins that are often associated to cell surfaces, which is the reason why Con A is extensively used as a tool in histochemical staining. Thus, it appeared of interest to further explore the potential of DCC by designing a dynamic library of constituents capable of binding to Con A. This would also provide a first step towards the generation of efficient substrates for other biological carbohydrate-binding targets.

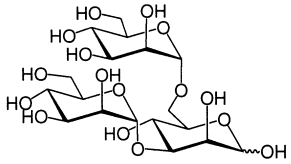
Implementation of the DCC approach: One may consider two procedures in the implementation of the DCC approach depending on whether library generation and screening are performed in a single step or in two steps, and defining two types of dynamic libraries.

a) *Adaptive combinatorial libraries*: The generation of the library constituents is conducted in a single step in presence of the target so that the library composition may adjust, leading to selection and amplification of the preferred substrate(s). The DCL may be real or virtual; screening by the target occurs in parallel with the reversible generation of the library constituents. This is the approach where the dynamic features are operative over the whole process, that is, the fully dynamic procedure representing the features analysed in detail in ref. [3].

b) *Pre-equilibrated dynamic combinatorial libraries (pDCLs)*: The constituents of the library are generated by reversible interconversion and equilibration in the absence of the target, which is added in a second step after reversibility has been stopped. This has the advantage that one may use reversible reactions which are not compatible with the presence of the target, but the process is not adaptive and no amplification of the preferred substrate can result. However, it is sufficient for lead generation, that is, the discovery of species having the desired activity; in its second phase, it amounts to the usual, static combinatorial chemistry approach in which an actual, real library is screened by the target. The resulting library may be termed pre-equilibrated or postdynamic combinatorial library (pDCL).

In the present work, both procedures have been implemented and will be described.

Design of the dynamic carbohydrate library: The binding site of Con A has been mapped by X-ray crystallography on several occasions and has been shown to be rather shallow, composed of a number of threonine and tyrosine hydroxy, aspartate carboxy, as well as asparagine and main-chain amide groups, capable of forming a network of hydrogen bonds to the natural substrate, the branched trimannoside unit $(Man)_3$.[19] The majority of these interactions are formed with the nonreducing, peripheral mannoside moieties of $(Man)_3$, rather than with the central mannose group, thus implying that the latter acts more or less as a linker between the interacting parts. It appeared that

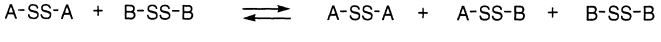


$(Man)_3$

it might be possible to mimic this interaction by a type of "bolasaccharide", that is, a structure containing two carbohydrate head groups joined by a spacer. If a reversible covalent bond could be introduced in the linker region, a library of dynamically interchanging species should then be produced, each carrying different linkers and carbohydrate head groups. Thus, by variation of the length of the linker region and of the carbohydrate head groups, new analogues of the natural trimannoside would be generated. In the present study, a dynamic ditopic carbohydrate library was designed, and its interaction with Con A was evaluated.[20]

To set up a versatile, yet robust dynamic library, an easily controllable, reversible reaction, capable of being operated under mild conditions, had to be selected. In addition, the reactivity needed to be compatible with the binding of the carbohydrates to Con A. The thiol – disulfide interconversion fulfils these prerequisites[21] and was already applied in the dynamic generation of an artificial tripeptide receptor.[9] Scheme 1 illustrates the scrambling process brought about by the disulfide interchange reaction.

The disulfide bond has several advantages when used in a dynamic combinatorial protocol. Firstly, disulfides undergo rapid interchange with thiols at moderate to high pH (≥ 7), but the bond formed is stable at low pH (< 5), thus allowing to lock/



Scheme 1. *Dynamic library generation using disulfide interchange. Left: starting library of two homodimers. Right: equilibrating dynamic library of dimeric constituents. Several homodimers may be introduced at the outset, of course, leading to a large number of heterodimers.*

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was born in Rosheim, France. He received his Doctorat-ès-Sciences in 1963 from the University of Strasbourg for work in the group of G. Ourisson. After a year as postdoctoral associate with R. B. Woodward at Harvard University, he joined the University Louis Pasteur in Strasbourg where he became professor of chemistry in 1970. In 1979 he was elected to the Chair of Chemistry of Molecular Interactions at the Collège de France in Paris. He has since been conducting research at both institutes. In 1987 he was awarded the Nobel Prize in Chemistry.

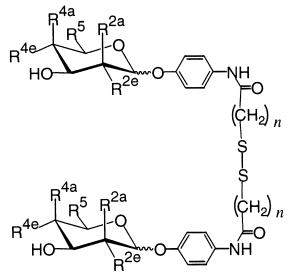
[*] *Members of the Editorial Advisory Board will be introduced to the readers with their first manuscript.*

unlock the dynamic process by a simple change in pH. Secondly, the reactivity of nonaromatic disulfides is reasonably similar, and the equilibrium constant is close to unity, that is, to isoenergetic behaviour. Thirdly, the interchange can be performed in aqueous environment under mild conditions. Finally, the reaction is highly chemoselective, so that only thiol – disulfide interchange occurs in the presence of other functional groups. In the light of the prerequisites for generating optimal dynamic libraries, these are all attractive features. In the present system with Con A as a receptor, the disulfide interchange could also be expected to occur without any interference with the protein, since the latter is devoid of disulfide bridges. This concern may, however, be of minor importance when considering other proteins, since protein disulfide bridges, often located in the interior of the macromolecule, are unlikely to be accessible to solution phase disulfides at low concentrations.

It has also been pointed out that, for maximising the effect of receptor binding on the real or virtual library constituents, it was desirable to have in hand an isoenergetic library and that one way to achieve this was to isolate the connective functional groups undergoing the reversible reaction from the varying recognition head groups by means of a spacer unit.[3]

The design of the initial carbohydrate library was based on considerations concerning the mimicking of the native trimannoside unit. Originally, a size of the linker as close as possible to the situation in trimannoside was aimed for. However, since the Con A binding site is rather shallow and freely accessible to the surrounding pool of solvent, it could be envisaged that a slightly longer, but sufficiently flexible chain could be used as well. Such a compound would then be able to fold into a conformation suitable for fitting into the site. Obviously, an excessively extended linker would result in poorer binding because of a higher entropic loss upon binding. Finally, the compounds **1** – **6** listed in Table 1 were chosen as a first approach to a library of carbohydrate homodimers, containing a disulfide bridge in the linker region. A phenylamido group was also introduced, primarily because it provided a chromophore for the subsequent HPLC analyses. Three hexopyranosides (D-mannose, D-glucose, D-galactose) and two pentopyranosides (L-arabinose, D-xylose) were used as carbohydrate head groups, and two different linkers that varied in length by one methylene group were examined.

Table 1. *Structures of the disulfide-linked carbohydrate dimers* ***1*** *–* ***6****.*



Compound[a]	α/β	R^{2a}	R^{2e}	R^{4a}	R^{4e}	R^5	n
1 (Man/Man)	α	OH	H	H	OH	CH_2OH	3
2 (Gal C_2/Gal C_2)	β	H	OH	OH	H	CH_2OH	2
3 (Gal C_3/Gal C_3)	β	H	OH	OH	H	CH_2OH	3
4 (Glc/Glc)	β	H	OH	H	OH	CH_2OH	2
5 (Ara/Ara)	β	H	OH	OH	H	H	2
6 (Xyl/Xyl)	β	H	OH	H	OH	H	2

[a] Man = D-mannose; Gal C_2 = D-galactose, $n = 2$; Gal C_3 = D-galactose, $n = 3$; Glc = D-glucose; Ara = L-arabinose; Xyl = D-xylose.

When analysing the generation and selection events in the presence of the receptor, a method for its (immediate) separation from the equilibrating library was required. For this reason, Con A immobilised on sepharose beads was used, thus allowing for simple filtration of the unbound species. Also, subsequent elution of the entity bound to the beads by acidifying the solution could easily be performed.

Upon scrambling of a homodimeric library (a vector of size n) of the kind used in this study, a library theoretically composed of all homo- and heterodimers should amount to a size of n^2, resulting in an $n \times n$ matrix. In the case of a library composed of heterodimers that are symmetrical, such that species A–SS–B equals B–SS–A, the size is reduced to the corresponding triangular matrix, consisting of $n(n+1)/2$ components (Scheme 2).

$$\begin{pmatrix} u_1 \\ u_2 \\ u_3 \\ \vdots \\ u_n \end{pmatrix} \quad \begin{pmatrix} a_{11} & a_{12} & a_{13} & \vdots & a_{1n} \\ a_{21} & a_{22} & a_{23} & \vdots & a_{2n} \\ a_{31} & a_{32} & a_{33} & \vdots & a_{3n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ a_{n1} & a_{n2} & a_{n3} & \vdots & a_{nn} \end{pmatrix} \quad \begin{pmatrix} a_{11} & a_{12} & a_{13} & \vdots & a_{1n} \\ 0 & a_{22} & a_{23} & \vdots & a_{2n} \\ 0 & 0 & a_{33} & \vdots & a_{3n} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & 0 & \vdots & a_{nn} \end{pmatrix}$$

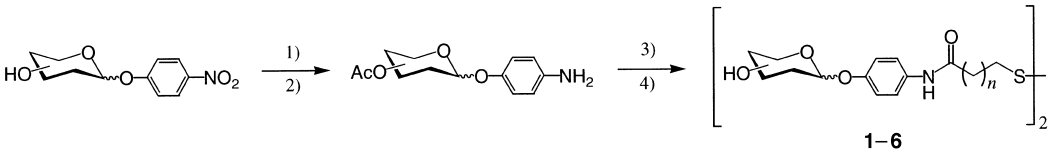
Scheme 2. *Size of dimeric dynamic libraries. Left: original set of* n *components that may combine two by two. Center: final equilibrated library of constituents representing all two-by-two contributions of* n *components; number of constituents* = n × n. *Right: case of a symmetrical library where* $a_{ij} = a_{ji}$*; number of different constituents* = n(n + 1)/2.

In the present study, such symmetrical libraries were employed, evaluated primarily for $n = 4$ and 6, thus resulting in a final library size of 10 and 21 constituents, respectively. Although these numbers are fairly small, compared to "classical" combinatorial libraries, the principle can easily be extended to larger libraries. For example, the use of a tetramer library composed of 20 different building blocks ($n = 20$) would yield up to $20^4 = 160\,000$ different, dynamically generated species.

Results

Generation of the library constituents and target binding

The libraries resulting from scrambling of the original dimers were generated by mixing dimers **1** – **6** (Scheme 3) together with an initiating reagent that is capable of reducing some of the disulfides to the corresponding thiols. Dithiothreitol (DTT) was found to be a good choice since this compound is efficiently oxidised to a stable 6-membered cyclic disulfide that may be expected not to take part in the scrambling of the library disulfides. Upon initiation/reduction, interconversion between



Scheme 3. *Synthesis of the bis-carbohydrates* ***1 – 6*** *(n = 1, 2; see Table 1). 1) Ac_2O, DMAP, CH_2Cl_2; 2) H_2, Pd/C, MeOH; 3) dithiodicarboxylic acid, EDC, CH_2Cl_2; 4) NaOMe, MeOH. DMAP = 4-(dimethylamino)pyridine; EDC = 1-ethyl-3-(3-dimethylamino)-propyl carbodiimide hydrochloride.*

the disulfides occurred, the rate of which was highly dependent upon the pH of the solution. At high pH (> 8), scrambling was achieved reasonably rapidly (within hours), whereas at low pH (< 5) no scrambling could be detected. On the other hand, without initiation, no significant scrambling occurred over two weeks. A similar pH dependence was recorded for the binding of the carbohydrates to the receptor, in which a pH close to neutral was preferable. A pH of 7.4 was chosen as a level at which a reasonable rate of scrambling could be obtained while receptor binding was not significantly affected. In the present system, the library had to be diluted for practical reasons, thus requiring a longer equilibration time as expected for a bimolecular reaction. Figure 1 illustrates schematically the carbohydrate scrambling process and the binding of the dimers to a receptor presenting two recognition sites.

In the case of the library of size 10 (10-library; $n = 4$), in the absence of any receptor, scrambling occurred smoothly at pH 7.4 from the original species (Figure 2a) generating all ten expected ditopic combinations in comparable amounts, as analysed by RP-HPLC (Figure 2b). When the receptor (in the form of sepharose-bound Con A) was added to the equilibrating pool, a shift in the concentrations of the different unbound constituents present in solution was recorded (Figure 2c). The fractions of some of the free species decreased, notably those of D-mannose-containing homo- and heterodimers. To characterise the species actually bound to the receptor, the Con A – sepharose beads were eluted by acidifying the solution to pH 4, and the composition of the eluate was analysed (Figure 2d). The assignment of the peaks to the different constituents of the scrambled libraries was made on the basis of the relative retention times. Clearly, the D-mannose homodimer was most efficiently bound to the lectin, and, to a lesser extent, the D-mannose-containing heterodimers. All other species in the equilibrating pool did not bind to Con A – sepharose and remained in solution. Thus, the receptor could be used to "fish out" the best bound species from the equilibrating pool.

When the receptor was present during the entire scrambling process, a slightly higher yield of the bis-mannoside could be recovered, compared to when it was added upon pre-equilibration. In the former case, the amount of bis-mannoside was approximately 2.1 times higher than the amounts of each of the D-mannose-containing heterodimers. In the latter situation, this ratio decreased to 1.5. This result is indicative of an adaptive effect where the lectin, to some extent, acts as a thermodynamic trap during scrambling.

The same behaviour, albeit less pronounced, was observed for the 21-library ($n = 6$; Figure 3). Due to the larger number of species resulting from the scrambling, difficulties in their separation were encountered with the system used, and not all components could be resolved under the HPLC conditions used (Figure 3a, b). This was particularly the case for the homodimer containing D-galactose and a linker with three methylene units (GalC_3), and the D-xylose homodimer (Xyl), which could not be distinguished by reversed-phase chromatography (Figure 3a). However, from cross-reference studies the resulting chromatographic separation pattern was, as could be expected, similar to the resulting 10-library. Upon elution from the Con A – sepharose and analysis of the eluate (Figure 3d), a much clearer picture could be attained: Mainly the D-mannose homodimer and, to some extent, the D-mannose-containing heterodimers were found to be bound, while all other library constituents remained in the eluate.

Discussion

The present results implement a number of the general features of the dynamic combinatorial chemistry/virtual combinatorial library (DCC/VCL) approach.[3] They indicate that it is possible to

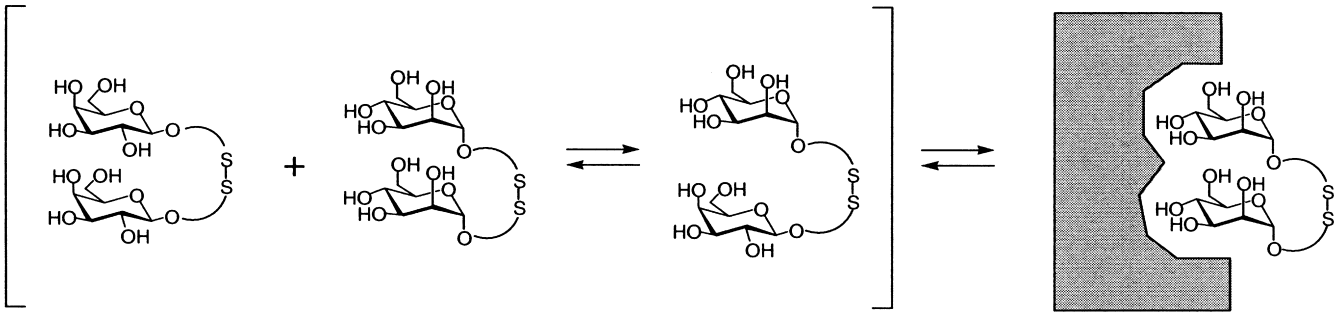


Figure 1. *Schematic representation of the scrambling process occurring in a mixture of disulfide-linked carbohydrate dimers, such as* ***1 – 6****, and of the binding of one of the constituents to a receptor with two recognition sites.*

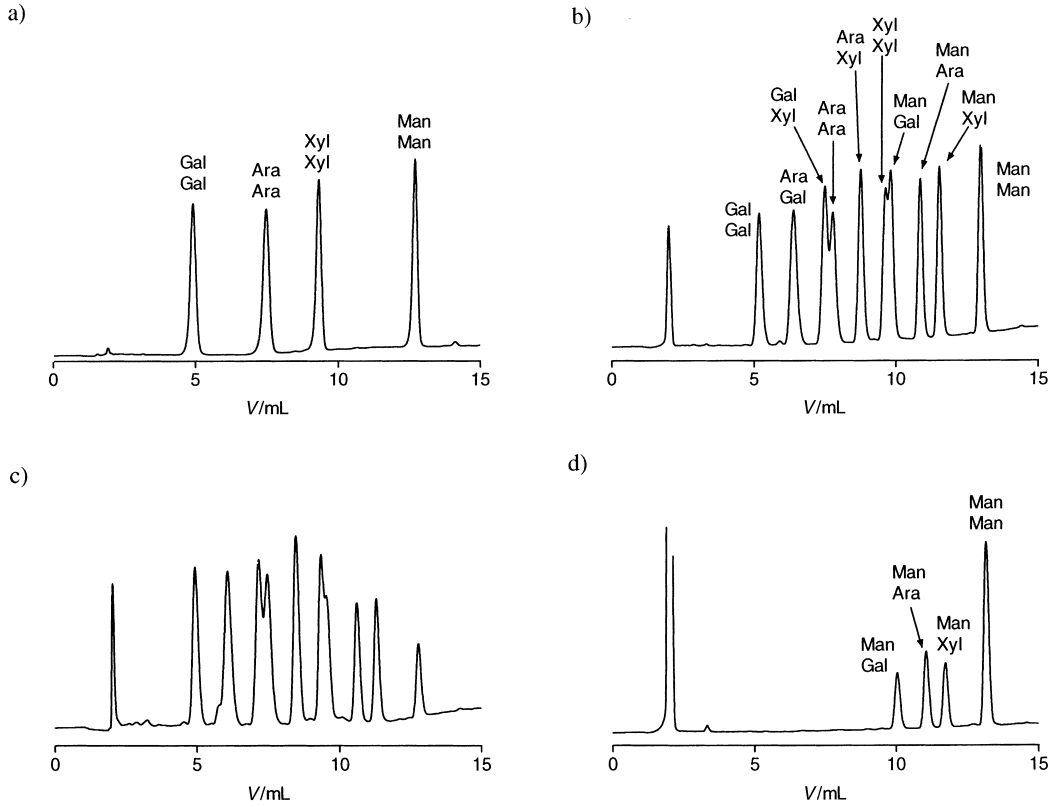


Figure 2. *RP-HPLC analysis of generation and screening of the 10-library (n = 4). a) Initial library; b) equilibrated dynamic library generated in the absence of Con A; c) equilibrated dynamic library generated in the presence of Con A; d) elution profile of species bound to Con A – sepharose.*

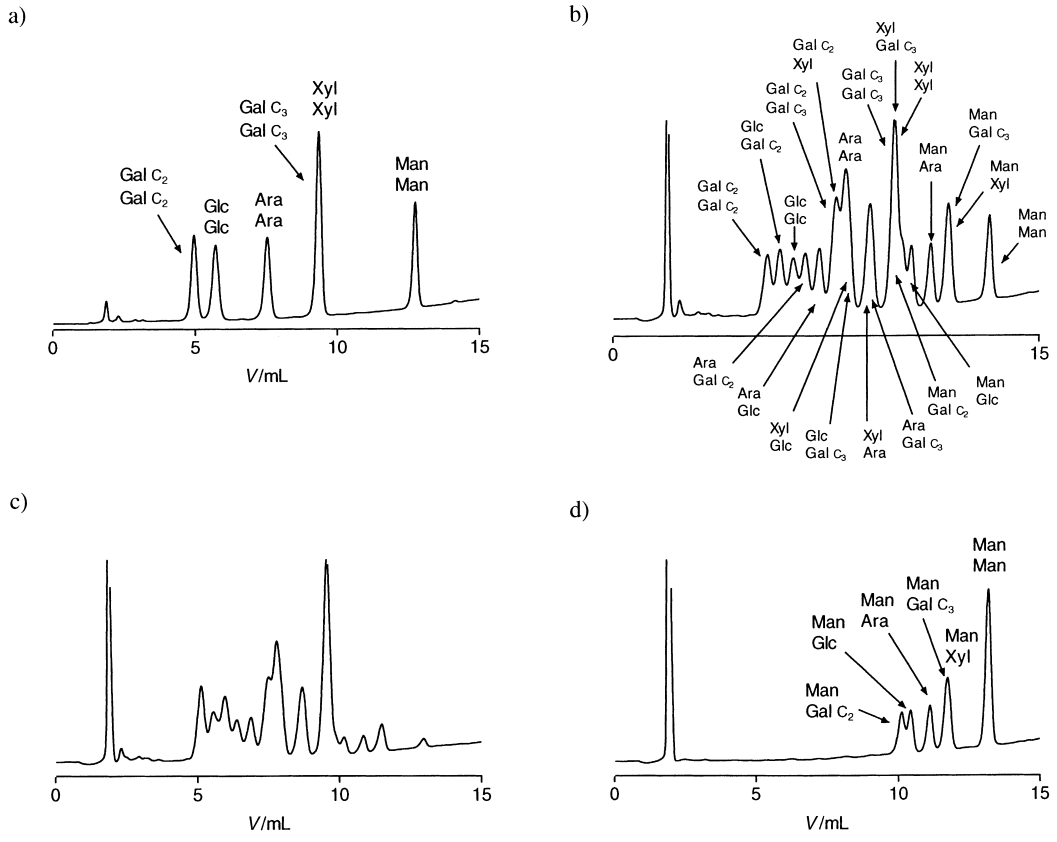


Figure 3. *RP-HPLC analysis of the 21-library (n = 6). a) Initial library; b) equilibrated dynamic library in the absence of Con A; c) equilibrated library in the presence of Con A; d) species bound to Con A – sepharose.*

generate a dynamic library of bis-carbohydrates based on reversible covalent disulfide bond formation between thiol-derivatised carbohydrate components in aqueous solution under mild conditions. The utilisation of the disulfide bond proved to be highly useful since it allowed for comfortable initiation of equilibration, and exchange could be stopped by simply acidifying the solution. This provides a simple start/stop mechanism that is easy to control. Other reversible bond types that can be envisaged in this respect, such as those present in imines, acetals, esters, or during alkene metathesis,[22] may offer useful alternatives to the disulfides, but in each case triggering and control must be chemically compatible with the targeted receptor.

Library generation and screening were accomplished following both the adaptive and the pre-equilibration procedures, although the amplification by thermodynamic trapping by the receptor was rather weak.

The results show that the DCL generated can be directly screened in situ by adding the targeted receptor to the equilibrating pool of library components, and that selective binding of specific constituents takes place. As long as the chemical interconversion does not interfere with the structure or function of the receptor and can be performed under sufficiently mild conditions, this offers a very convenient method for the rapid screening of a large number of compounds directly in one step. In addition, a shift in equilibrium can be expected resulting in amplification of the species most strongly bound to the receptor at the expense of the others. A clear preference for binding of the mannoside dimer **1** and to a lesser extent of the mannoside heterodimers was observed. However, no clear selectivity was found between the shorter C_2 and the longer C_3 spacers, probably due to the fact that this represents only a small relative change in length of the bridge linking the two carbohydrate groups.

The present DCL is also an isoenergetic library, whose different constituents are about equally populated. This is an important feature that may in general be achieved, as is the case here, by introducing a spacer between the variable unit(s), interacting with the receptor site, and the constant functional group(s) effecting the reversible connective process.[3] It maximises in principle the effect of receptor binding on the composition of the equilibrating library.

Conclusion

The results described here further implement the general concepts of the DCC/VCL approach,[1–3] in particular by exploring carbohydrate recognition groups, by performing external control of the interconversion process (through pH change), by achieving isoenergetic behaviour, by making use of an immobilised receptor for screening the library, and by applying both the fully dynamic, adaptive and the pre-equilibrated, postdynamic procedures.

In comparison to other combinatorial techniques, such as parallel/multiple synthetic protocols and encoded solution phase techniques, this methodology provides in principle access to easily controlled solution phase libraries. The size of the libraries increases rapidly if trimers, tetramers, etc., are envisaged, resulting in numbers of constituents well on a par with existing static chemical libraries. In conjunction with the in situ receptor-binding technique, such libraries can easily be screened, and the resulting bound antagonists/inhibitors identified. Extension to other library components, reversible processes, and biological targets is being actively pursued.

Experimental Section

Materials and instruments: Immobilised concanavalin A (Sigma, C-9017, 15 mg protein per mL of packed gel), bis-dithiodiacetic acid (Acros), bis-dithiodipropionic acid (Acros), and all other chemicals were of highest commercial purity and used as received. Optical rotations were measured with a Perkin–Elmer model 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC200 spectrometer at 298 K. Electrospray ionisation and fast atom bombardment mass spectra were determined by the Service de spectrométrie de masse at the Institut de Chimie, Université Louis Pasteur. HPLC analyses were performed with a Hewlett–Packard series HP 1100 chromatograph, equipped with a quaternary gradient pump, and a diode array detector.

Synthesis of the library components: The carbohydrate dimers were synthesised from the corresponding peracetylated 4-aminophenyl glycosides by condensation with the bis-dithio diacids, followed by deacetylation under standard Zemplén conditions (NaOMe/MeOH) (Scheme 3).[23] The 4-nitrophenyl glycosides used as starting materials were commercial products (Fluka or Sigma). The 4-aminophenyl derivatives were all obtained from the commercial 4-nitrophenyl glycosides following the same procedure described hereafter for one of them.

4-Aminophenyl *α*-L-2,3,4-tri-*O*-acetyl-arabinopyranoside: The 4-nitrophenyl glycoside (500 mg) was slurried in dichloromethane (2.5 mL) and acetic anhydride (2.5 mL). A catalytic amount of dimethylaminopyridine (DMAP) was added and the reaction was allowed to proceed under argon at ambient temperature overnight. Following washing (water, HCl (aq.), $NaHCO_3$ (aq.), water), the product solution was concentrated and dried in vacuo. The product was subsequently dissolved in methanol (40 mL), palladium on charcoal was added (5%, 140 mg), and reduction was performed under H_2 for 4 h. After removing of the catalyst by filtration, concentration and drying, the pure product was obtained as a white foam in 93% yield. $[\alpha]_D^{20} = +29$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 2.06$ (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.15 (s, 3H, Ac), 3.52 (br. s, 2H, NH_2), 3.68 (dd, 1H, $J_{4,5e} = 1.8$, $J_{5a,5e} = 13.0$ Hz, H-5e), 4.10 (dd, 1H, $J_{4,5a} = 3.7$ Hz, H-5a), 4.89 (d, 1H, $J_{1,2} = 6.7$ Hz, H-1), 5.11 (dd, 1H, $J_{2,3} = 9.2$, $J_{3,4} = 3.5$ Hz, H-3), 5.30 (m, 1H, H-4), 5.40 (dd, 1H, H-2), 6.61 (d, 2H, $J_{o,m} = 8.8$ Hz, H-*ortho*), 6.85 (d, 2H, H-*meta*); ^{13}C NMR (50 MHz, $CDCl_3$, 298 K): $\delta = 170.4$, 170.2, 169.5, 149.8, 142.5, 118.8, 116.0, 100.6, 70.1, 69.2, 67.5, 63.1, 21.0, 20.8, 20.8; MS (FAB, positive mode): *m/z*: 367.3 ($[M]^+$, calcd: 367.2); elemental analysis calcd for $C_{17}H_{21}NO_8$ (%): C 55.58, H 5.76, N 3.81; found: C 55.31, H 5.75, N 3.70.

4-Aminophenyl *β*-D-2,3,4-tri-*O*-acetyl-xylopyranoside: yield 94%; $[\alpha]_D^{20} = -27$ ($c = 0.5$, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta =$ 2.06 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.09 (s, 3H, Ac), 3.45 (dd, 1H, $J_{4,5a} =$ 8.2, $J_{5a,5e} = 12.0$ Hz, H-5a), 3.52 (br. s, 2H, NH_2), 4.20 (dd, 1H, $J_{4,5e} =$ 4.9 Hz, H-5e), 4.97 (d, 1H, $J_{1,2} = 6.1$ Hz, H-1), 4.97–5.03 (m, 3H, H-2, H-3, H-4), 6.61 (d, 2H, $J_{o,m} = 8.8$, H-*ortho*), 6.83 (d, 2H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD, 25 °C): $\delta = 171.7$, 151.2, 144.5, 119.6, 117.7,

101.7, 73.3, 72.6, 70.4, 63.2, 20.8; MS (FAB, positive mode): *m/z*: 367.1 ($[M]^+$, calcd: 367.2); elemental analysis calcd for $C_{17}H_{21}NO_8$ (%): C 55.58, H 5.76, N 3.81; found: C 55.47, H 5.99, N 3.76.

4-Aminophenyl β-D-2,3,4,6-tetra-*O*-acetyl-galactopyranoside: quant. yield; $[\alpha]_D^{20} = +4.6$ ($c = 10.0$, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): $\delta = 1.98$ (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.15 (s, 3H, Ac), 3.55 (br.s, 2H, NH_2), 3.97 (m, 1H, H-5), 4.12 (dd, 1H, $J_{5,6e} = 6.3$ Hz, H-6e), 4.22 (dd, 1H, $J_{5,6a} = 7.0$ Hz, H-6a), 4.85 (d, 1H, $J_{1,2} = 7.9$ Hz, H-1), 5.06 (dd, 1H, $J_{2,3} = 10.5$, $J_{3,4} = 3.4$ Hz, H-3), 5.41 (m, 2H, H-2, H-4), 6.58 (d, 2H, $J_{o,m} = 8.8$ Hz, H-*ortho*), 6.84 (d, 2H, H-*meta*); ^{13}C NMR (50 MHz, $CDCl_3$, 25 °C): $\delta = 170.4$, 170.2, 169.5, 150.0, 142.7, 119.0, 115.9, 101.2, 76.5, 71.0, 68.9, 67.1, 61.4, 20.7; MS (FAB, positive mode): *m/z*: 439.3 ($[M]^+$, calcd: 439.2); elemental analysis calcd for $C_{20}H_{25}NO_{10}$ (%): C 54.67, H 5.73, N 3.19; found: 54.47, H 5.66, 3.06.

The other 4-aminophenyl derivatives had properties that were in agreement with the data reported in the literature: 4-aminophenyl β-D-2,3,4,6-tetra-*O*-acetyl-glucopyranoside,[24] 4-aminophenyl α-D-2,3,4,6-tetra-*O*-acetyl-mannopyranoside.[25]

The carbohydrate dimers were obtained by the following procedure: A suspension of 4-aminophenyl glycoside (0.57 mmol), 1-ethyl-3-(dimethylaminopropyl)-carbodiimide (0.63 mmol), and bis-dithio diacid (0.29 mmol) was stirred for 4 h at room temperature under argon in dichloromethane (5 mL). Following washing (water, $NaHCO_3$ (aq.), water), and concentration of the organic phase, the product was purified by chromatography. Subsequent deprotection yielded the pure carbohydrate dimer.

Bis-mannoside 1: The intermediate product was purified by flash chromatography (SiO_2; dichloromethane/acetone (8:2, v/v)); yield 67%. $[\alpha]_D^{20} = +94$ ($c = 0.5$, MeOH/H_2O (1:1, v/v)); ^{1}H NMR (200 MHz, CD_3OD/D_2O (1:1, v/v), 25 °C): $\delta = 2.06$ (m, 4H, β-CH_2), 2.47 (t, 4H, $J =$ 7.2 Hz, γ-CH_2), 2.77 (t, 4H, $J = 7.0$ Hz, α-CH_2), 3.53 – 3.66 (m, 2H, H-5), 3.66 – 3.78 (m, 6H, H-4, H-6a, H-6e), 3.88 (dd, 2H, $J_{3,4} = 9.2$ Hz, H-3), 3.98 (dd, 2H, $J_{2,3} = 3.4$ Hz, H-2), 5.40 (d, 2H, $J_{1,2} = 1.5$ Hz, H-1), 7.05 (d, 4H, $J_{o,m} = 8.9$ Hz, H-*ortho*), 7.44 (d, 4H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD, 25 °C): $\delta = 172.0$, 153.1, 133.0, 121.4, 116.7, 99.1, 73.9, 71.0, 70.6, 67.0, 61.3, 37.5, 34.7, 24.8; MS (ES, positive mode): *m/z*: 745.5 ($[M+H]^+$, calcd: 744.2); elemental analysis calcd for $C_{32}H_{44}N_2O_{14}S_2$ (%): C 51.60, H 5.95, N 3.76; found: 51.84, H 6.18, N 3.54.

Bis-galactoside 2: Purification by preparative TLC (Chromatotron, Al_2O_3; dichloromethane/1% MeOH (v/v)), yield 86%. $[\alpha]_D^{20} = -46$ ($c = 0.2$, MeOH/H_2O (1:1, v/v)); ^{1}H NMR (200 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 2.81$ (t, 4H, $J = 6.6$ Hz, β-CH_2), 3.06 (t, 4H, $J = 7.1$ Hz, α-CH_2), 3.66 – 3.83 (m, 10H, H-2, H-3, H-5, H-6a, H-6e), 3.97 (d, 2H, $J = 2.6$ Hz, H-4), 4.89 (d, 2H, $J_{1,2} = 7.6$ Hz, H-1), 7.09 (d, 4H, $J_{o,m} = 9.0$ Hz, H-*ortho*), 7.40 (d, 4H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta =$ 171.7, 154.3, 132.4, 122.5, 117.0, 101.5, 75.4, 72.9, 70.7, 68.6, 60.7, 35.7, 33.6; MS (ES, positive mode): *m/z*: 739.3 ($[M+Na]^+$, calcd: 716.2); elemental anaylsis calcd for $C_{30}H_{40}N_2O_{14}S_2$ (%): C 50.27, H 5.62, N 3.91; found: C 50.03, H 5.66, N 3.76.

Bis-galactoside 3: Purification by preparative TLC (Chromatotron, Al_2O_3; dichloromethane/0.5% MeOH (v/v)), yield 36%. $[\alpha]_D^{20} = -43$ ($c = 0.08$, MeOH/H_2O (1:1, v/v)); ^{1}H NMR (200 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 2.06$ (m, 4H, β-CH_2), 2.49 (t, 4H, $J = 6.5$ Hz, γ-CH_2), 2.79 (t, 4H, $J = 6.7$ Hz, α-CH_2), 3.61 – 3.84 (m, 10H, H-2, H-3, H-5, H-6a, H-6e), 3.96 (br.s, 2H, H-4), 4.89 (d, 2H, $J_{1,2} = 7.0$ Hz, H-1), 7.09 (d, 4H, $J_{o,m} =$ 9.0, H-*ortho*), 7.39 (d, 4H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 173.4$, 154.2, 132.5, 122.6, 117.0, 101.5, 75.4, 72.9, 70.7, 68.6, 60.8, 37.4, 34.8, 24.8; MS (ES, positive mode): *m/z*: 767.2 ($[M+Na]^+$, calcd: 744.2); elemental analysis calcd for $C_{32}H_{44}N_2O_{14}S_2$ (%): C 51.60, H 5.95, N 3.76; found: 50.78, H 6.13, N 3.52.

Bis-glucoside 4: Purification by flash chromatography (SiO_2; dichloromethane/acetone (8:2, v/v)), yield 67%. $[\alpha]_D^{20} = -40$ ($c = 0.3$, MeOH/H_2O (1:1, v/v)); ^{1}H NMR (200 MHz, CD_3OD/D_2O (4:1, v/v), 25 °C): $\delta = 2.80$ (t, 4H, $J = 6.3$ Hz, β-CH_2), 3.05 (t, 4H, $J = 6.9$ Hz, α-CH_2), 3.53 – 3.66 (m, 8H, H-2, H-3, H-4, H-5), 3.71 (dd, 2H, $J_{5,6a} = 4.9$, $J_{6a,6e} = 12.1$ Hz, H-6a), 3.89 (dd, 2H, $J_{5,6e} = 1.4$ Hz, H-6e), 4.94 (d, 2H, $J_{1,2} = 7.0$ Hz, H-1), 7.07 (d, 4H, $J_{o,m} = 9.0$ Hz, H-*ortho*), 7.38 (d, 4H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD/D_2O (4:1, v/v), 25 °C): $\delta = 171.1$, 154.2, 132.8, 121.8, 116.9, 101.1, 76.5, 76.2, 73.4, 69.8, 61.0, 35.8, 33.7; MS (ES, positive mode): *m/z*: 739.2 ($[M+Na]^+$, calcd: 716.2); elemental analyis calcd for $C_{30}H_{40}N_2O_{14}S_2$ (%): C 50.27, H 5.62, N 3.91; found: C 50.22, H 5.72, N 3.77.

Bis-arabinoside 5: Purification by flash chromatography (SiO_2; dichloromethane/acetone (8:2, v/v)), yield 42%. $[\alpha]_D^{20} = -6$ ($c = 0.2$, MeOH/H_2O (1:1, v/v)); ^{1}H NMR (200 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 2.83$ (s, 4H, $J = 6.7$ Hz, β-CH_2), 3.08 (s, 4H, α-CH_2), 3.70 – 3.87 (m, 6H, H-2, H-5a, H-5e), 3.93 – 3.99 (m, 4H, H-3, H-4), 4.84 (d, 2H, $J_{1,2} =$ 6.9 Hz, H-1), 7.03 (d, 4H, $J_{o,m} = 9.1$ Hz, H-*ortho*), 7.38 (d, 4H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 171.8$, 154.1, 132.3, 122.6, 117.1, 101.5, 72.4, 70.6, 68.2, 66.2, 35.7, 33.6; MS (ES, positive mode): *m/z*: 657.0 ($[M+H]^+$, calcd: 656.2); elemental analysis calcd for $C_{28}H_{36}N_2O_{12}S_2$ (%): C 51.21, H 5.53, N 4.27; found: C 51.86, H 5.26, N 4.32.

Bis-xyloside 6: Purification by preparative TLC, (Chromatotron, Al_2O_3; dichloromethane/1% MeOH (v/v)), yield 63%. $[\alpha]_D^{20} = -21$ ($c = 0.3$, MeOH/H_2O (1:1, v/v)); ^{1}H NMR (200 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 2.84$ (t, 4H, $J = 6.8$ Hz, β-CH_2), 3.09 (t, 4H, $J = 6.4$ Hz, α-CH_2), 3.40 – 3.76 (m, 8H, H-2, H-3, H-4, H-5e), 4.00 (dd, 2H, $J_{4,5} = 4.6$, $J_{5a,5e} =$ 11.4 Hz, H-5a), 4.94 (d, 2H, $J_{1,2} = 7.2$ Hz, H-1), 7.07 (d, 4H, $J_{o,m} = 9.0$ Hz, H-*ortho*), 7.42 (d, 4H, H-*meta*); ^{13}C NMR (50 MHz, CD_3OD/D_2O (1:1), 25 °C): $\delta = 171.8$, 153.9, 132.5, 122.6, 117.0, 101.4, 75.8, 73.1, 69.3, 65.3, 35.7, 33.6; MS (ES, positive mode): *m/z*: 679.2 ($[M+Na]^+$, calcd: 656.2); elemental analysis calcd for $C_{28}H_{36}N_2O_{12}S_2$ (%): C 51.21, H 5.53, N 4.27; found: C 51.15, H 5.42, N 4.10.

Generation and analysis of adaptive combinatorial libraries: To a solution of carbohydrate dimers **1** – **6** (10 μM each) in buffer (900 μL, 100 mM Na phosphate, 50 μM $MnCl_2$, 50 μM $CaCl_2$, pH 7.4) was added a suspension of Con A – sepharose (50 μL, ca. 5 nmol Con A), and dithiothreitol solution (50 μL, 20 mM). After equilibration at ambient temperature for two weeks, the suspension was filtered (Millipore, Amicon YM-100), and the sepharose particles resuspended in buffer (400 μL). The bound carbohydrate dimers were released by addition of HCl (100 μL, 1 M), and after renewed filtration, the solutions were analysed by RP-HPLC.

Generation and analysis of pre-equilibrated combinatorial libraries: The same protocol as for the adaptive libraries was used, with the exception that Con A – Sepharose (50 μL) was added after the equilibration period. The resulting suspension was then allowed to further equilibrate during 1 h at room temperature, and the bound species were eluted and analysed as described above.

HPLC analyses: The library compositions were analysed by RP-HPLC (LiChroCART 250-4 column, LiChrospher 100, RP-18, 5 μm; Merck). The elution was monitored with a diode array detector set at 255 nm. Injection volume: 20 μL. MeOH – H_2O gradient: 0 – 3 min, 35% MeOH; 3 – 15 min, 35 →65% MeOH; 15 – 16 min, 65 →35% MeOH; 16 – 20 min, 35% MeOH.

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