Chiral selection and chiral induction by the use of regiospecifically di-(or poly)substituted cyclodextrins

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Abstract - Regiospecific bifunctionalization of β-cyclodextrins
is achieved by use of rigid difunctional modifying reagents.
Successive (two step) nucleophilic substitution on a regio-
specifically capped cyclodextrin leads to a β-cyclodextrin
derivative having two different functional groups on regio-
specific positions. Such compounds behave as artificial enzymes
and receptors. A typical example of an artificial receptor is
the cyclodextrin having amino and carboxyl groups on the regio-
specific prim, prim positions, which recognizes free (unmodified)
amino acids quite strongly in a neutral aqueous solution.
A-(Modified B_6)-B[ω-amino(ethylamino)]-β-Cyclodextrin accelerates
conversion from keto acids to L-amino acids in 98/2, 98/2 and
95/5 L/D ratio for phenylglycine, Phe and Trp, respectively.

1. GENERAL STRATEGY FOR ENZYME MIMICKS BY THE USE OF MODIFIED CYCLODEXTRINS

Cyclodextrins, cyclic oligomers (hexamer, heptamer, octamer, etc) of glucose,
were easily obtainable from potato starch. Each cyclodextrin has a torus
shape cavity inside the molecule, which is surrounded by axial C-H bonds of
glucoses, making the environment hydrophobic. From the torus, hydroxyl
groups are sticking out, making the molecule water soluble (see Fig. 1).
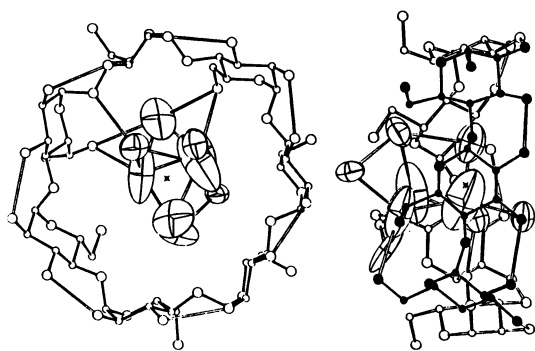

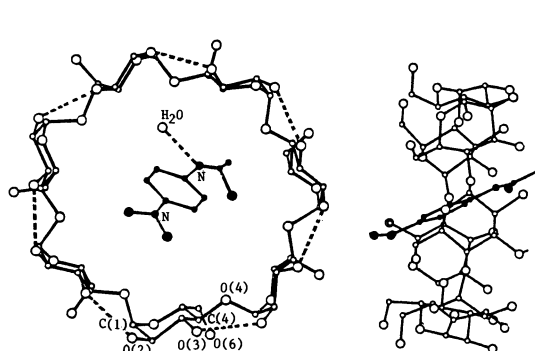Fig.1.The crystallographic structure of β-cyclodextrin (ref. 1)

Fig.2. The crystallographic structure of the inclusion complex of β-cyclodextrin with p-nitrophenylacetoamide (ref.2)

Most probably water molecules penetrate into the cavity in an aqueous
solution, as shown in X-ray crystallography. These water molecules are not
satisfactorily hydrogen bonded, in other words they are destabilized to some
extent, being readily driven out when a more suitable guest — a hydrophobic
guest — comes to contact. Again this situation is well demonstrated by the
X-ray crystallography (Fig. 2). The guest best accommodated into the cyclo-
dextrin cavity, therefore, is to be best recognized by the C-H wall of the
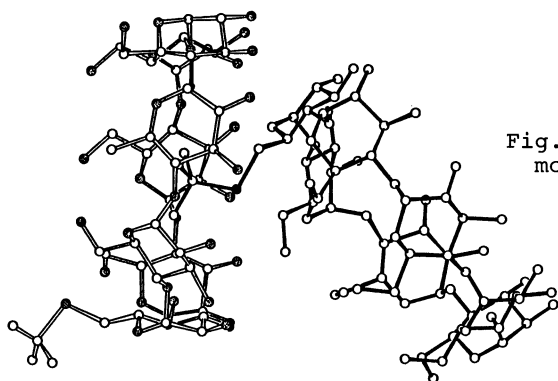cavity in a way of best van der Waals surface contact.

Fig. 3. The crystallographic structure of
mono-6-t-butyl-S-β-cyclodextrin (ref. 3)

The situation still holds for modified cyclodextrins, as typically shown by
X-ray crystallography of primary t-BuS-β-cyclodextin (Fig. 3).

An extremely simple strategy for enzyme mimick is then to be drawn that an
efficient catalytic site mimicking an enzyme catalytic site is to be intro-
duced directly onto the "rim" of a certain cyclodextrin torus. A number of
examples were reported in literature, which well mimick rather simple enzyme
catalysis — enormous acceleration and rather strict substrate specificity
of pattern recognition type (size and shape recognition)(ref. 4a, b) (see
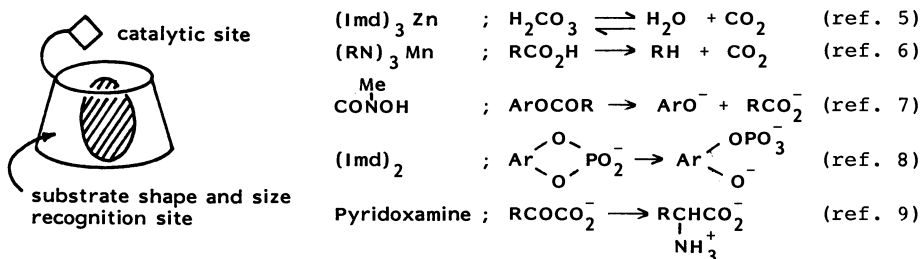Fig. 4).

$(Imd)_3 Zn$	; $H_2CO_3 \rightleftharpoons H_2O + CO_2$	(ref. 5)
$(RN)_3 Mn$	; $RCO_2H \longrightarrow RH + CO_2$	(ref. 6)
$\overset{Me}{\underset{|}{CONOH}}$	; $ArOCOR \longrightarrow ArO^- + RCO_2^-$	(ref. 7)
$(Imd)_2$	; $Ar\overset{O}{\underset{O}{<}}PO_2^- \longrightarrow Ar\overset{OPO_3^-}{\underset{O^-}{<}}$	(ref. 8)
Pyridoxamine	; $RCOCO_2^- \longrightarrow R\underset{\underset{NH_3^+}{|}}{C}HCO_2^-$	(ref. 9)

Fig. 4. Enzyme mimicks by use of modified cyclodextrins

2. CHIRAL SELECTION VIA CENTRIPETAL CHIRAL RECOGNITION AIDED BY HYDROPHOBIC BINDING

Guest molecules having hydrophobic group(s) to be strictly recognized by the
cyclodextrin cavity often possess hydrophobic binding energy as large as 3
Kcal (ref. 10). Under the circumstances, additional host guest-recognition
interaction is operating for other functional groups, X, Y and Z. The situa-
tion is well understood by looking from the opposite side of R as shown in
Fig. 5b, to which the Newman projection may be appropriately applied. Chiral
selection is then to be carried out by the complementary grouping X' Y' and
Z' existing along the enzyme wall. A typical intuitive example is selective
recognition of hydrophobic 1-amino acid derivatives from the d-isomer by
α-Chymotrypsin (ChT). The bulky SMe grouping prefers to accommodate the least
bulky H and two OH groupings interact with CONH and NHCO via hydrogen bonding.
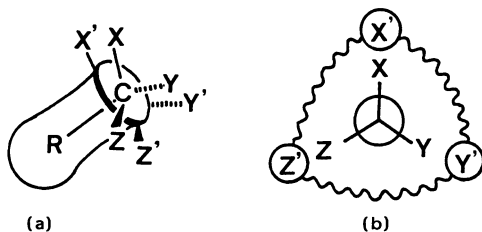

(a)

(b)

Fig. 5. The chiral selection by host
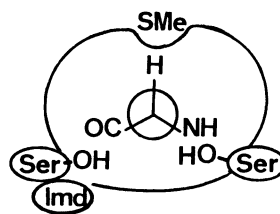guest-recognition interactions

Fig. 6. The selective recognition
by α-Chymotrypsin

As a result, d- and l-isomers are bound to ChT with practically the same association constants. Only the difference, significant difference though, between l- and d-isomer binding is the activation of Ser[195] by His[57], but the latter does not exist for the other Ser. This leads to ready hydrolysis of the l-isomer, while d-isomer acts as a competitive inhibitor. In this particular example, X', Y' and Z' define a clockwise direction, which keeps chiral cavity even in the absence of a chiral guest. Whenever a couple of d/l stereoisomers interacts with the host chiral cavity, recognition is achieved via "centripetal" fashion. This chiral selection mode is in a sharp and interesting contrast to the "centrifugal" selection mode often applied to organic synthesis (Fig. 7).
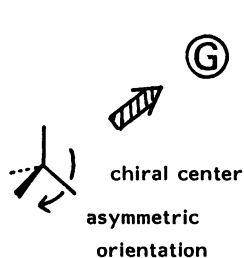

chiral center
asymmetric
orientation

Fig. 7. The "centri-
fugal" selection
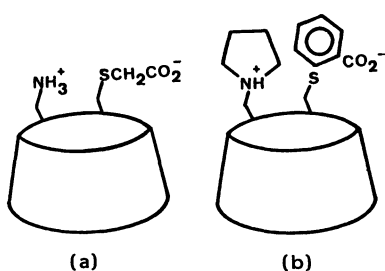mode

(a)   (b)

Fig. 8. Hosts for chiral
separation of l- and d-
amino acids

TABLE 1.   Ratio of Disubsti-
tuted CD/ Mono CD.

Reagent	Di CD	Mono CD	Di CD/ Mono CD
	26	16	1.62
	12	28	0.423

* [β-CD] = 2.55 × 10⁻³ M

Assuming that this "centripetal" chiral recognition is also important in organic chemistry, one can design a host molecule for chiral selection without much difficulty. A simple-minded example is a host for chiral separation of l- and d- amino acids, an appropriate molecular framework is easily to be drawn after ChT chiral selection mode. Thus, disubstituted cyclodextrins (Fig. 8) are chosen as promising candidates.

A,B- and A,C- capped cyclodextrins were powerful starting materials to reach the synthetic goal (ref. 11). As shown in Fig. 9, straight-forward syntheses provide the target compounds Fig. 8a and b in satisfactory yields (ref. 12).
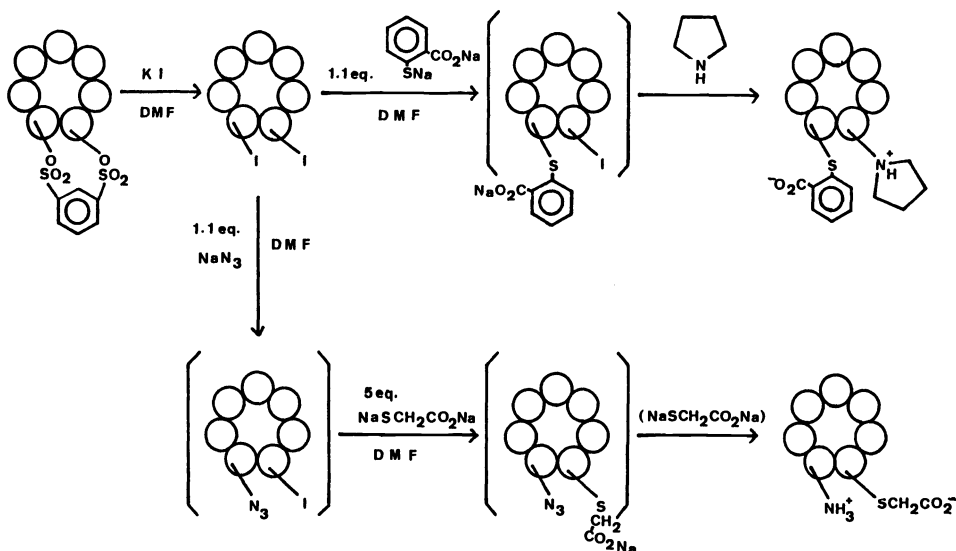

Fig. 9.   The synthetic scheme

Capping technique (based on "molecular measure" principle) is extremely important for the synthesis of regiospecifically di- (tri-, tetra- etc) substituted cyclodextrins, by which a certain target regioisomer is obtainable much more efficiently and in much better yields than routine, laborious successive substitution (ref. 13) (see Table 1).

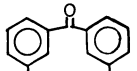TABLE 2. Regioisomer Distribution
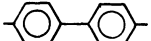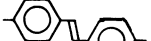of Capping Reactions.
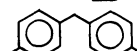
Capping Reagent	AB	AC	AD
(structure)	97	5	0
(structure)	0	94	6
(structure)	0	7	93
(structure)	0	8	92
(structure)	0	66	34

TABLE 3. Chiral Recognition of Tryptophan
by Artificial Receptor.

CD	Guest	K_{ass}	CRC
$A,B-(SCH_2CO_2^-)(NH_4^+)$	L-Trp	14.7	
	D-Trp	10.8	0.73
$A,B-(S\text{-}C_6H_4\text{-}CO_2^-)(N\text{-ring})$	L-Trp	42.5	
	D-Trp	54.0	1.27

Results of 1- and d-amino acid separation in neutral aqueous solution at
room temperature are listed in Table 3. Apparently, chiral separation was
poor in using the hydrophilic chiral host (Fig 8a). Two findings are new and
worthy to be noted. 1) ΔH is reasonably negative but is, in most part, com-
pensated by the accompanying unfavorable entropy change 2) ΔH is much less
negative than expected. The latter observation is interpreted by the con-
sideration of very polar local environment, giving very weak polar interac-
tion based on the equation;

$$U = z_1z_2/\varepsilon r$$

The interpretation is verified by the observation of considerably larger
negative ΔH for chiral host having much more hydrophobic local environment,
where d- and l-separation become reasonably efficient. The finding of
entropy-enthalpy compensation seems to be due to loss of rotational freedom
of the guest bound to the chiral host. Observed NMR relaxation time con-
firmed the remaining freedom when a monofunctional guest was bound to a
monofunctional CD.

Therefore, an ideal amino acid receptor may be designed as a quadruple recog-
nition type, having a strongly hydrophobic local site.

The present chiral recognition is already reasonably strong, giving a prom-
ise of ready d/l amino acid separation in water, when the host is immobilized
for column chromatography.

3. CHIRAL INDUCTION VIA CENTRIPETAL CHIRAL RECOGNITION

When a prochiral grouping is fixed in a certain reasonable way in the "cen-
tripental chiral cavity", efficient chiral induction is to be expected. This
is the case for aminotransfer reaction in which an amino acid is produced
from a keto acid by the help of pyridoxamine. The molecular plane of the
pyridoxamine moiety is strictly recognized by the enzyme wall through cen-
tripetal interaction as depicted in Fig. 10 for Aspartate aminotransferase
(ref. 14).

A keto acid, then, is bound to pyridoxamine to give an extended molecular
plane still fixed by the centripetal recognition by the enzyme wall, leaving
an ω-NH$_2$ grouping free which is located just above the Schiff-base molecular
plane. The prototropy reaction followed, leading to 1-amino acid formation.
If correct, a very similar situation is gained by use of A,B-disubstituted-
β-CD. Thus, B$_6$-enzyme model, together with its regioisomer (which may be
called artificial isozyme) were prepared via straight-forward synthesis
(ref. 15) (Fig. 11), but separation of these were not easy at all. A self-
selection rule was found powerful in this particular example, which enabled
efficient separation of A,B- and B,A-regioisomers. The pyridoxamine regio-
isomers were converted to the corresponding pyridoxal mixture, to which
L-phenylalanine was applied. Interestingly, the A,B isomer was converted
back to the pyridoxamine preferentially, while the B,A isomer remained
in a form of the pyridoxal. By simple separation of the amine from the
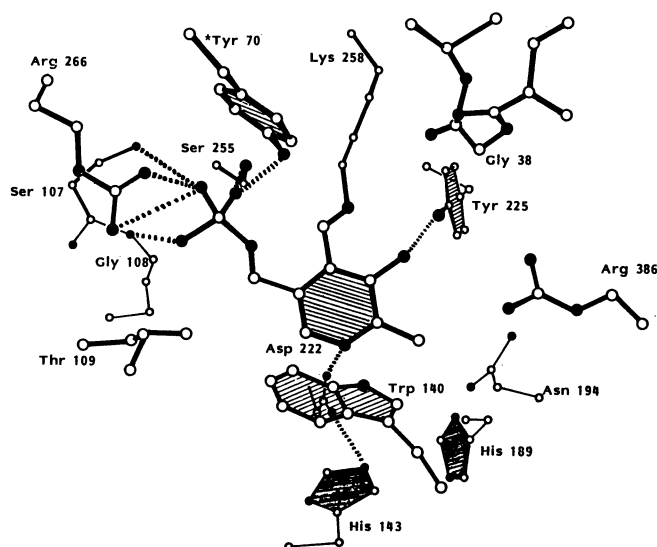aldehyde, the regioisomer separation was successful (see Fig. 12).

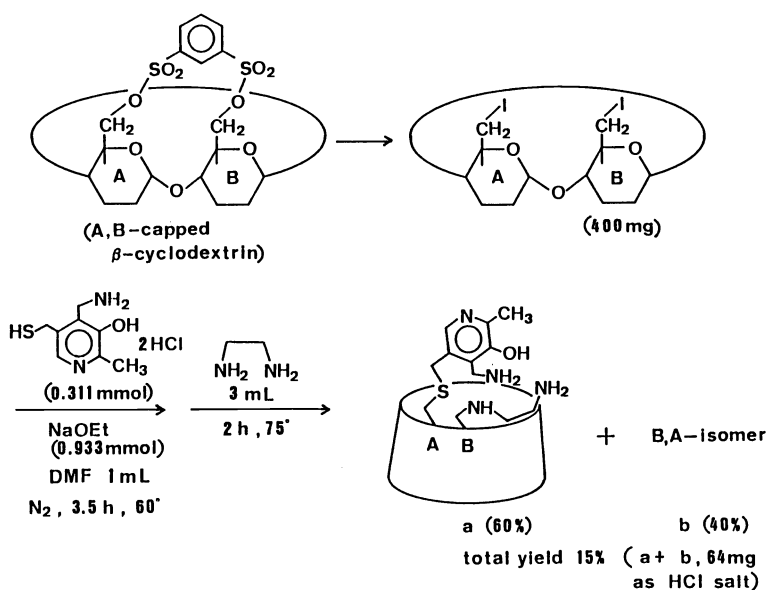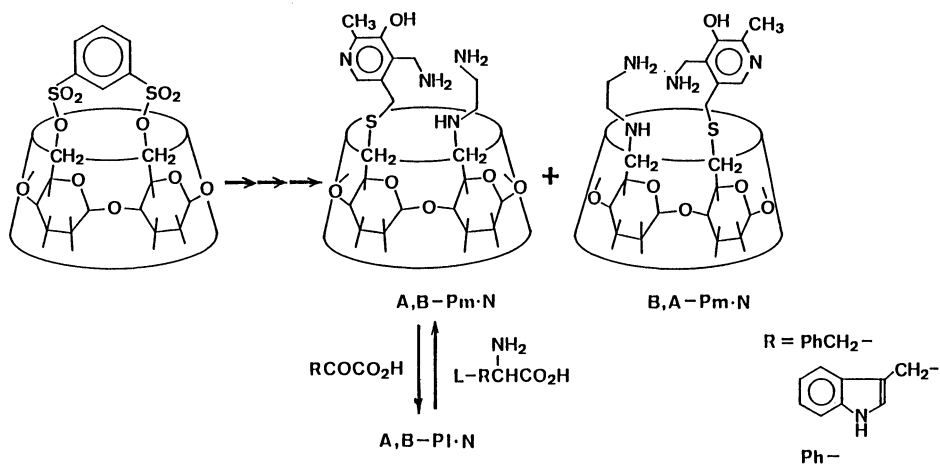Fig. 10. The active site of Aspartate aminotransferase

Fig. 11. The synthetic scheme of B$_6$-enzyme model

Fig. 12. The self-selection of artificial isozymes

TABLE 4. Chiral Induction in Amino
Acid Formed Via Artificial B_6
Catalysis.

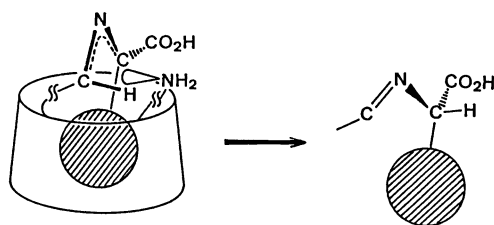| $RCOCO_2H$	L/D
$R = CH_2\phi$	98/2
$= CH_2$ (indolylmethyl)	95/5
$= \phi$	98/2

Fig. 13. The chiral induction of
amino acid

The artificial B_6 enzyme of centripetal chiral recognition capacity then con-
verted several keto acids to the corresponding L-amino acids in 90-96 % ee
(Table 4). The B,A-isomer, as expected, provided D-amino acids in similarly
selective fashion.

The chiral induction is best interpreted by stereospecific approach of the
ω-NH_2 grouping on the B-glucose ring to the B_6-Schiff-base molecular plane
which is fixed by covalent bond to the A-glucose ring, hydrophobic binding
of an aromatic moiety and hydrophilicity of the CO_2^- grouping (Fig. 13).

The mechanism is well supported by the large accelerating effect of the
ω-NH_2 grouping (ca. × 10), strong binding of the hydrophobic guests to the
cavity and large acceleration therefrom.

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