Table S1 Oligonucleotide primers used for mutagenesis insertion

Primer	primer sequence
E141Q_foward	5′-GTGCCGTCATCAAGAAGCAAAGCTACTTTGGGG-3′
E141Q_reverse	5′-CCCCAAAGTAGCTTTGCTTCTTGATGACGGCAC-3′
E141A_forward	5′-GTGCCGTCATCAAGAAGGCAAGCTACTTTGGGG-3′
E141A_reverse	5′-CCCCAAAGTAGCTTGCCTTCTTGATGACGGCAC-3′
E141D_forward	5′-GTGCCGTCATCAAGAAGGACAGCTACTTTGGGG-3′
E141D_reverse	5′-CCCCAAAGTAGCTGTCCTTCTTGATGACGGCAC-3′
E141N_forward	5′-GTGCCGTCATCAAGAAGAACAGCTACTTTGGGG-3′
E141N_reverse	5′-CCCCAAAGTAGCTGTTCTTCTTGATGACGGCAC-3′
E162Q_forward	5′-GGATTGATGCAGGTTCAACCTAACACGCGG-3′
E162Q_reverse	5′-CCGCGTGTTAGGTTGAACCTGCATCAATCC-3′
E162A_forward	5′-GGATTGATGCAGGTTGCACCTAACACGCGG-3′
E162A_reverse	5′-CCGCGTGTTAGGTGCAACCTGCATCAATCC-3′
E162D_foward	5′-GGATTGATGCAGGTTGACCCTAACACGCGG-3′
E162D_reverse	5′-CCGCGTGTTAGGGTCAACCTGCATCAATCC-3′
E162N_forward	5′-GGATTGATGCAGGTTAACCCTAACACGCGG-3′
E162D_reverse	5′-CCGCGTGTTAGGGTTAACCTGCATCAATCC-3′
D226N_forward	5′-GTATCCAGGAGCTACAAACTCCTATGGCCGCCCG-3′
D226N_reverse	5′-CGGGCGGCCATAGGAGTTTGTAGCTCCTGGATAC-3′
D226A_forward	5′-GTATCCAGGAGCTACAGCATCCTATGGCCGCCCG-3′
D226A_reverse	5′-CGGGCGGCCATAGGATGCTGTAGCTCCTGGATAC-3′

The mutation sites are underlined.

Table S2 Crystallization conditions for five structures

Structure name	Protein	Reservoir solution	Soaking solution /soaking time	Observed ligands at a substrate binding site
-	SeMet -labeled Ra-ChiC$_{cat}$	8.3% (w/v) PEG3350 1.3% (v/v) isopropanol 33.3 mM $CaCl_2$ 33.3 mM HEPES (pH7.5)	none	-
WT	WT Ra-ChiC$_{cat}$	5% (w/v) PEG8000 167 mM $(NH_4)_3$ citrate/ ammonium hydroxide (pH 8.5)	none	Glycerol ×1
WT-NAG2	WT Ra-ChiC$_{cat}$	8.3% (w/v) PEG3350 1.3% (v/v) isopropanol 33.3 mM $CaCl_2$ 33.3 mM HEPES (pH7.5)	Reservoir solution containing 40 mM $(NAG)_2$ /7 hours	$(NAG)_2$ ×2
E141Q-NAG4	E141Q Ra-ChiC$_{cat}$	11.1% (w/v) PEG3350 1.8% (v/v) isopropanol 44.4 mM $CaCl_2$ 44.4 mM HEPES (pH7.5)	Reservoir solution containing 40 mM $(NAG)_4$ /30 hours	$(NAG)_4$ ×1
E162Q-HEPES	E162Q Ra-ChiC$_{cat}$	11.1% (w/v) PEG3350 1.8% (v/v) isopropanol 44.4 mM $CaCl_2$ 44.4 mM HEPES (pH7.5)	None	HEPES ×1
E162Q-NAG2	E162Q Ra-ChiC$_{cat}$	8.9% (w/v) PEG8000 8.9% (v/v) PEG400 44.4 mM $MgCl_2$ 44.4 mM Tris-Cl (pH8.5)	Reservoir solution containing 40 mM $(NAG)_2$ /20 hours	$(NAG)_2$ ×2

Table S3 Data collection statistics for MAD phasing

	SeMet-substituted Ra-ChiC_{cat} -MAD			
	peak	edge	remote (high)	remote (low)
Data collection				
Wavelength (Å)	0.9789	0.9730	0.9641	0.9950
Space group	$P6_122$	$P6_122$	$P6_122$	$P6_122$
Resolution (Å)	2.20 (2.28-2.20)	2.20 (2.28-2.20)	2.20 (2.28-2.20)	2.20 (2.28-2.20)
Completeness (%)	99.9 (100.0)	99.9 (100.0)	99.9 (100.0)	99.9 (100.0)
^{a} R_{merge} (%)	11.8 (52.8)	11.4 (55.2)	11.5 (58.0)	10.5 (57.7)
$\langle I/\sigma(I)\rangle$	46.6 (9.7)	46.2 (9.3)	45.4 (8.7)	46.9 (8.6)

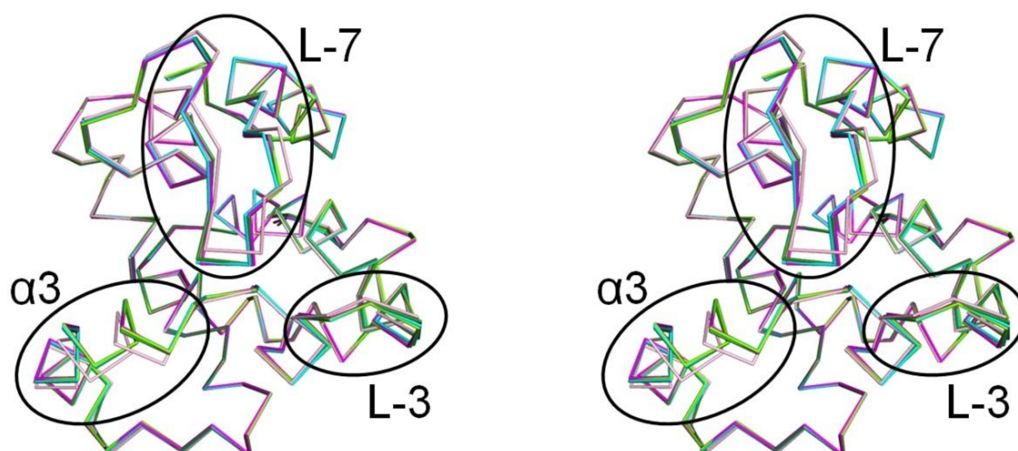

Figure S1. Stereo diagram of superposed Ra-ChiC$_{cat}$ structures are shown as wire models. WT, WT-NAG2, E141Q-NAG4, E162Q-HEPES, and E162Q-NAG2 structures are shown in dark green, light green, cyan, light magenta, and magenta, respectively. α3, L-3, and L-7 regions in which conformational changes are observed are indicated by ellipses.

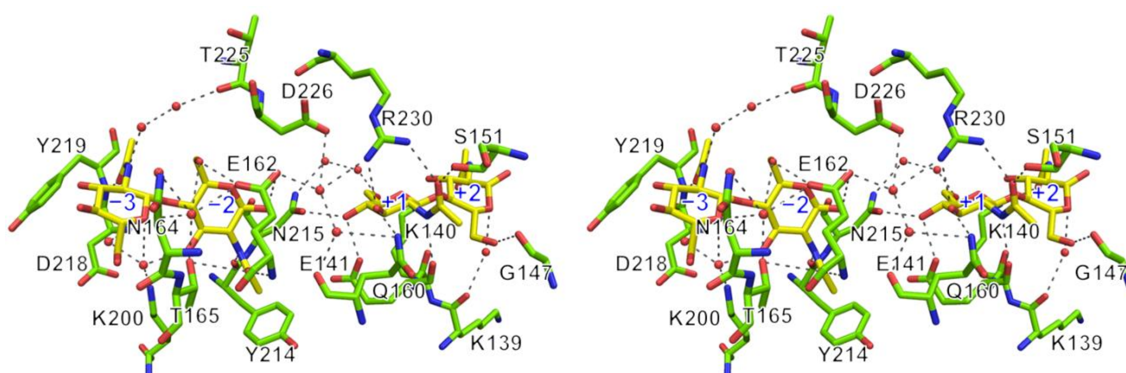

Figure S2. Stereo diagrams showing the NAG recognition mechanisms observed in the WT-NAG2 structure. The bound NAG residues are shown in yellow. Hydrogen bonds are shown as gray dashed lines.

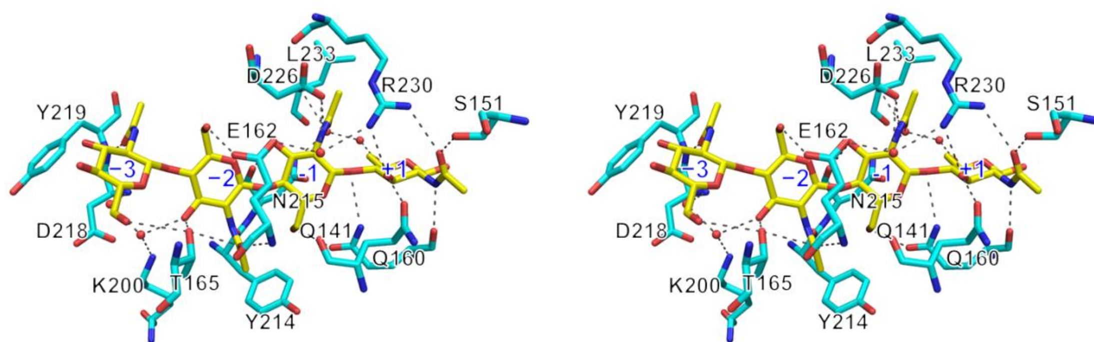

Figure S3. Stereo diagrams showing the NAG recognition mechanisms observed in the E141Q-NAG4 structure. The bound NAG residues are shown in yellow. Hydrogen bonds are shown as gray dashed lines.

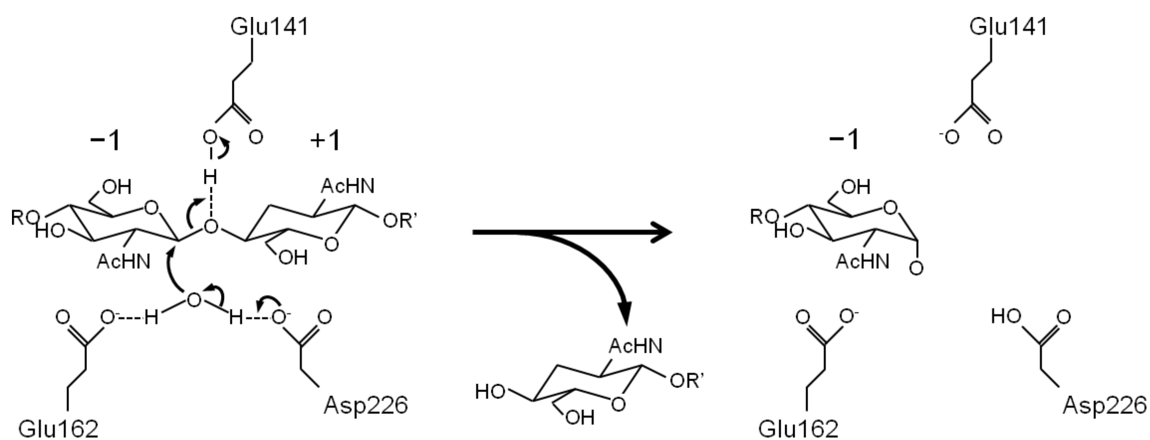

Figure S4. Catalytic mechanism of Ra-ChiC proposed based on structural and mutagenic analyses.