Electronic Supplementary Information (ESI)

Improving the catalytic ability of peptide-based artificial glycosidase through tyrosine strategy

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Table S1 Peptides for the construction of glycosidase-like catalysts

Peptide assemblies	Peptide sequence
A_4-E_3	Fmoc-AAAAEEE-$CONH_2$
YA_3-E_3	Fmoc-YAAAEEE-$CONH_2$
A_3Y-E_3	Fmoc-AAAYEEE-$CONH_2$
YA_2Y-E_3	Fmoc-YAAYEEE-$CONH_2$

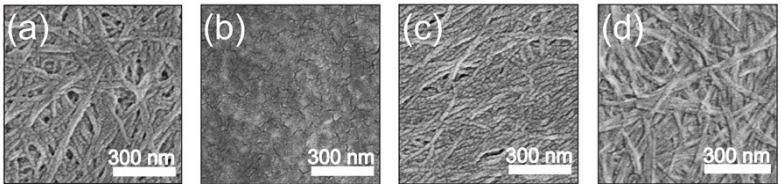

Fig. S1 SEM images of (a) A_4-E_3, (b) YA_3-E_3, (c) A_3Y-E_3 and (d) YA_2Y-E_3.

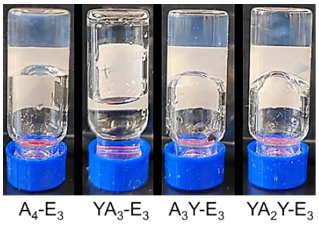

Fig. S2 Photographic images of peptide assemblies

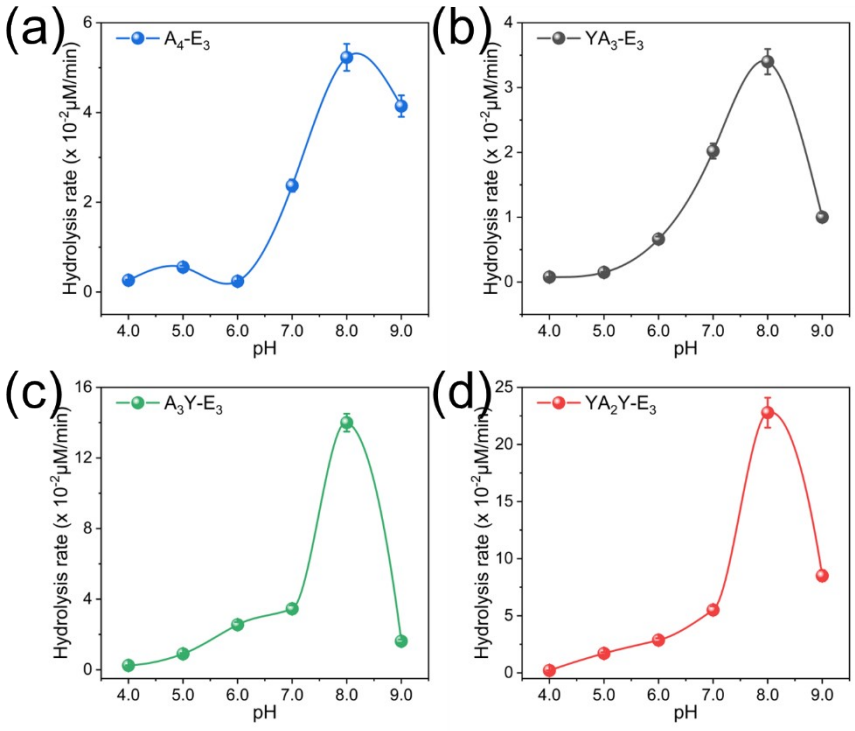

Fig. S3 pH-*V* profiles for the hydrolysis of *p*-NPG catalyzed by peptide assemblies, (a)

A_4-E_3, (b) YA_3-E_3, (c) A_3Y-E_3 and (d) YA_2Y-E_3.

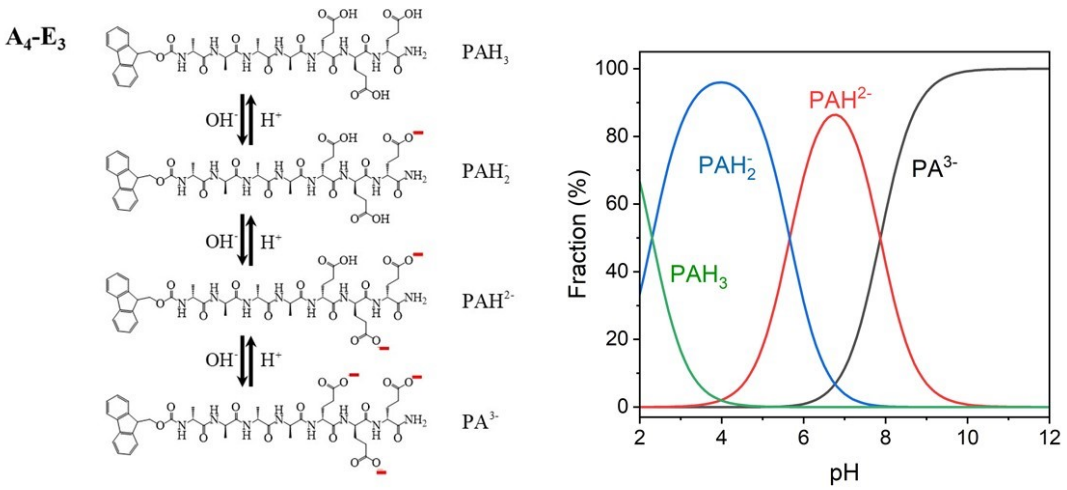

Fig. S4 Relative abundance of different protonated and deprotonated species of A_4-E_3.

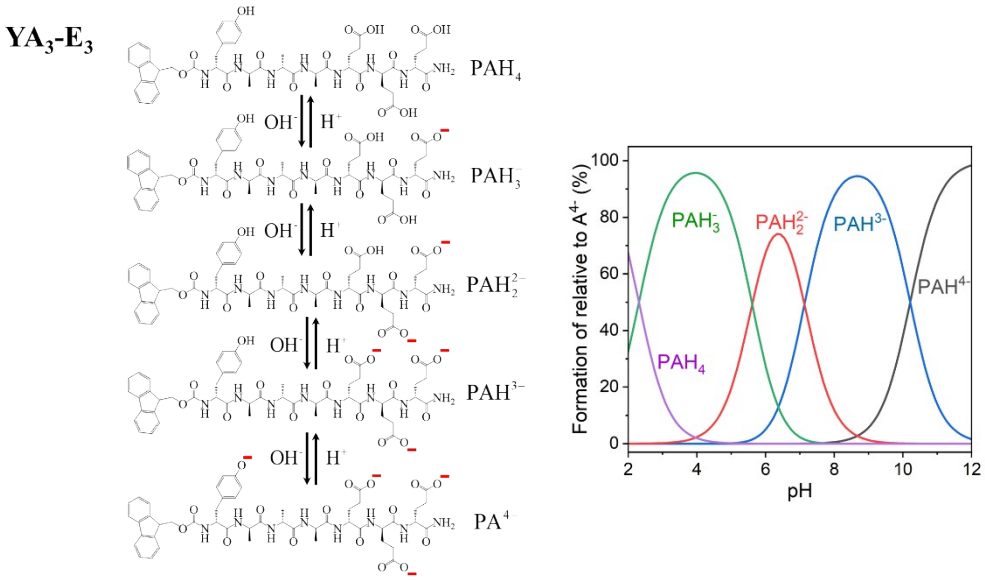

Fig. S5 Relative abundance of different protonated and deprotonated species of YA_3-E_3.

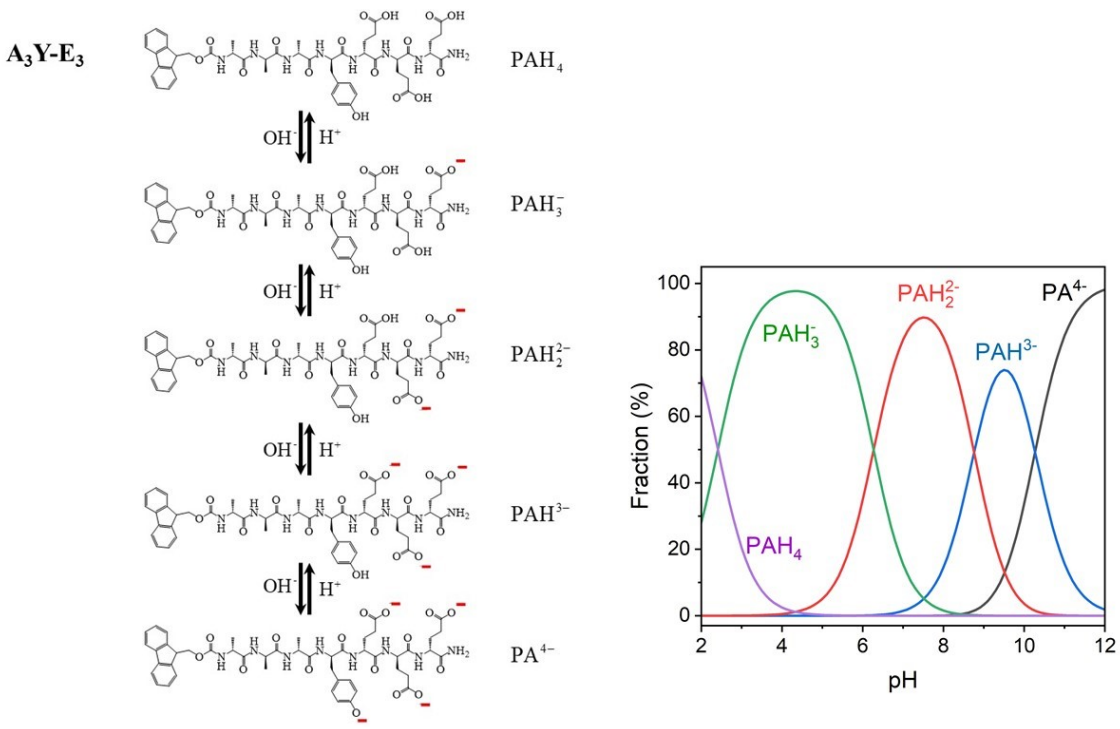

Fig. S6 Relative abundance of different protonated and deprotonated species of $A_3Y\text{-}E_3$.

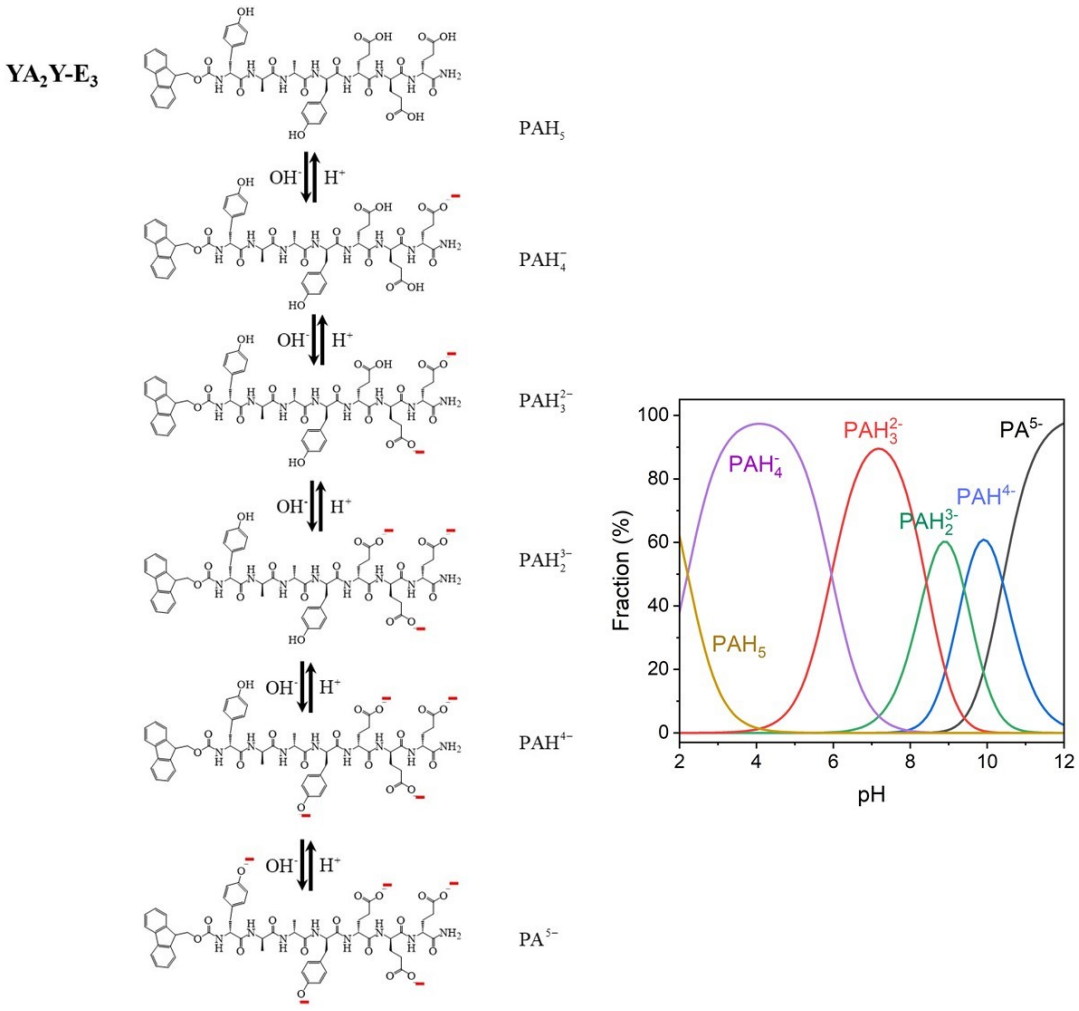

Fig. S7 Relative abundance of different protonated and deprotonated species of YA_2Y-E_3.

Table S2 The protonation state of peptide catalysts under pH 8 [a]

A_4-E_3	PAH_3	PAH_2^-	PAH^{2-}	PA^{3-}		
Fraction (%)	0	0.19	42.49	57.32		
YA_3-E_3	PAH_4	PAH_3^-	PAH_2^{2-}	PAH^{3-}	PA^{4-}	
Fraction (%)	0	0.046	11.58	87.82	0.55	
A_3Y-E_3	PAH_4	PAH_3^-	PAH_2^{2-}	PAH^{3-}	PA^{4-}	
Fraction (%)	0	1.56	83.8	14.56	0.08	
YA_2Y-E_3	PAH_5	PAH_4^-	PAH_3^{2-}	PAH_2^{3-}	PAH^{4-}	PA^{5-}

Fraction (%)	0	0.62	70.74	27.52	1.12	0

[a] The gray shadow indicates catalytically active states

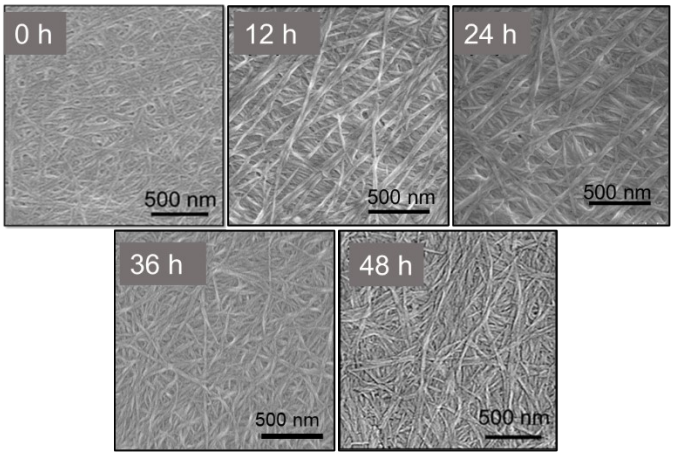

Fig. S8 SEM images of CL-YA_2Y-E_3 at different cross-linking time.

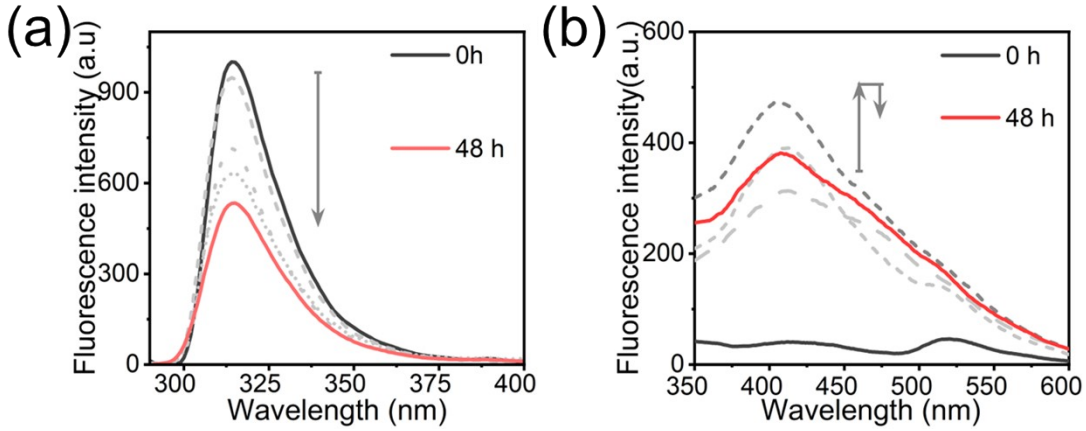

Fig. S9 (a) Fluorescence emission spectrum of the YA_2Y-E_3 solution (1 mM/mL) under 274 nm excitation. (b) Fluorescence emission spectrum of the YA_2Y-E_3 solution (1 mM/mL) under 320 nm excitation.

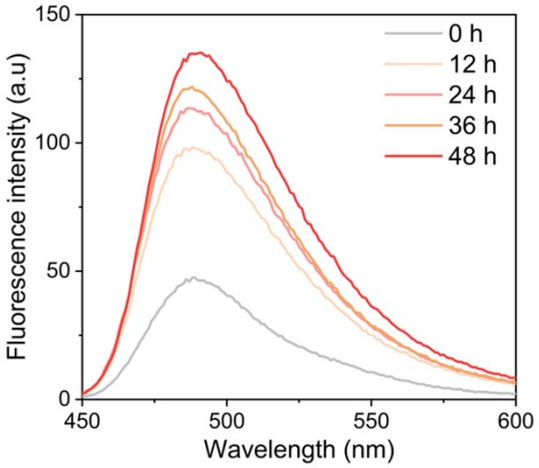

Fig. S10 Th-T fluorescence spectrum of the CL-YA_2Y-E_3 at different cross-linking time.

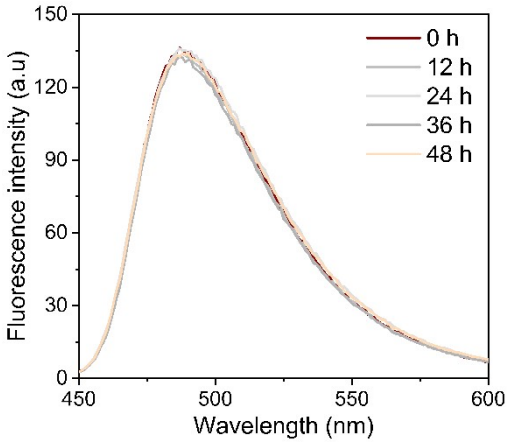

Fig. S11 Th-T fluorescence spectrum of the CL-A_4-E_3 at different cross-linking time.

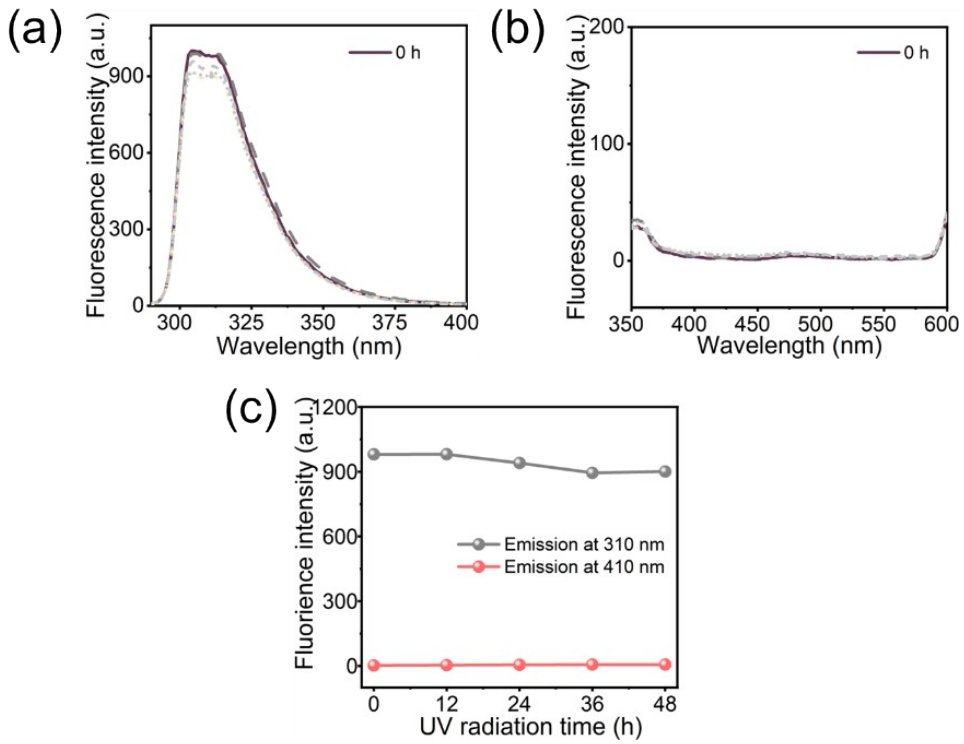

Fig. S12 (a) Fluorescence emission spectrum of the A_4-E_3 solution (1 mM/mL) under 274 nm excitation. (b) Fluorescence emission spectrum of the A_4-E_3 solution (1 mM/mL) under 320 nm excitation. (c) Fluorescence intensity of emission at 274 nm and 320 nm varies with time.

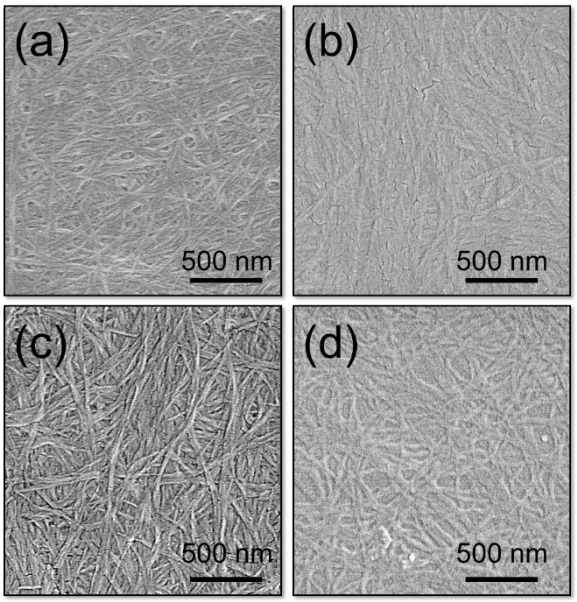

Fig. S13 SEM images of YA_2Y-E_3 before (a) and after (b) the p-NPG hydrolysis reactions. SEM images of CL-YA_2Y-E_3 before (c) and after (d) the p-NPG hydrolysis reactions.

Table S3 Comparison of YA_2Y-E_3, CL-YA_2Y-E_3 with some reported glycosidase mimics in *p*-NPG (**1**) and 4-nitrophenyl α-$_D$-glucoside hydrolysis (**2**).

Catalyst	Temperature	Substrate	K_m (mM)	V_{max} (μM/min)	k_{cat} (x10^{-3} s^{-1})	k_{cat}/K_m (M^{-1} min^{-1})	Substrate	Reaction time	Specific activity (μmol/h/mg)	Reference
YA_2Y-E_3	30 °C	**1**	1.64	0.29	0.0032	0.117	**2**	96 h	66.2	This work
CL-YA_2Y-E_3			2.59	0.657	0.0073	0.168			123.4	
6^A-(*R*)-cyanohydrin CD (**19**)	59 °C	**1**	5.36		0.0012	0.013	**2**	48 h	32.28	1
6^A,6^D-dicarboxylic acid β-CD (**29**)	59 °C		n.d.		0.0170				45.0	
Catalyst 1	59 °C	**1**	13.40		0.000334	0.0015	**2**	48 h	8.28	2
Binuclear Copper(II) Complexes 12	30 °C	**1**	213		0.07	0.00204	**2**	48 h	96.70	3
Cyclodextrins 13	30 °C	**1**	80.30		0.0142	0.0106	**2**	48 h	38.62	4
Dicyanohydrin-β-cyclodextrin (1)	59 °C	**1**	4.14		0.0103	0.149				5
Dicyanohydrin-α-cyclodextrin (2)			6.34	0.33	0.0093	0.0880				
Cyclodextrin Derivatives (11)	59 °C	**1**	7.60		0.0014	0.0111				6
Compound 2	59 °C	**1**	2.90		0.0012	0.0123	**2**	48 h	3.83	7

Reference

1. J. Bjerre and M. Bols, *European Journal of Organic Chemistry*, 2010, **2010**, 3487-3500.
2. C. Rousseau, N. Nielsen and M. Bols, *Tetrahedron Letters*, 2004, **45**, 8709-8711.
3. S. Striegler, N. A. Dunaway, M. G. Gichinga, J. D. Barnett and A.-G. D. Nelson, *Inorganic Chemistry*, 2010, **49**, 2639-2648.
4. Y. Zhou, C. M. Pedersen and M. Bols, *Tetrahedron Letters*, 2013, **54**, 2458-2461.
5. F. Ortega-Caballero, J. Bjerre, L. S. Laustsen and M. Bols, *Journal of Organic Chemistry*, 2005, **70**, 7217-7226.
6. F. Ortega-Caballero, C. Rousseau, B. Christensen, T. E. Petersen and M. Bols, *Journal of the American Chemical Society*, 2005, **127**, 3238-3239.
7. J. Bjerre, T. H. Fenger, L. G. Marinescu and M. Bols, *European Journal of Organic Chemistry*, 2007, **2007**, 704-710.