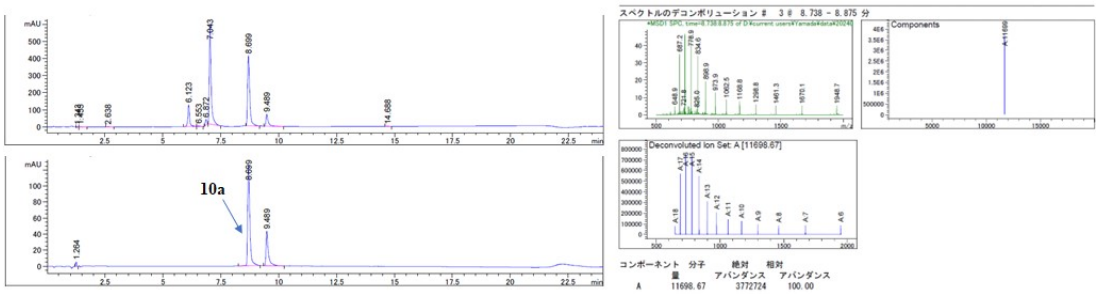
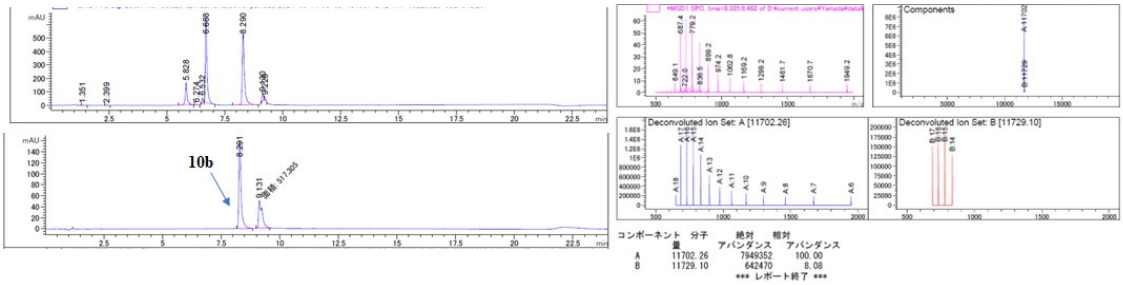
1. **Supplementary Figures (Figure S1–S6)**
2. **Supplementary Tables (Table S1–S3)**
3. **Supplementary methods**

1. Supplementary Figures (Figure S1–S6)



Sequence of **10a**	Calculated MS	Observed MS
Cy3ACCGAGACCTGAAGTGGTGGGA ACTGCGCTGAAAAA	11694	11698

Figure S1 Result of the LC/MS analysis of **10a** (Table 1, entry 2).



Sequence of **10b**	Calculated MS	Observed MS
Cy3ACCGAGACCTGAAGTGGU(F)GG GAACTGCGCTGAAAAA	11698	11702

Figure S2 Result of the LC/MS analysis of **10b** (Table 2, entry 1).

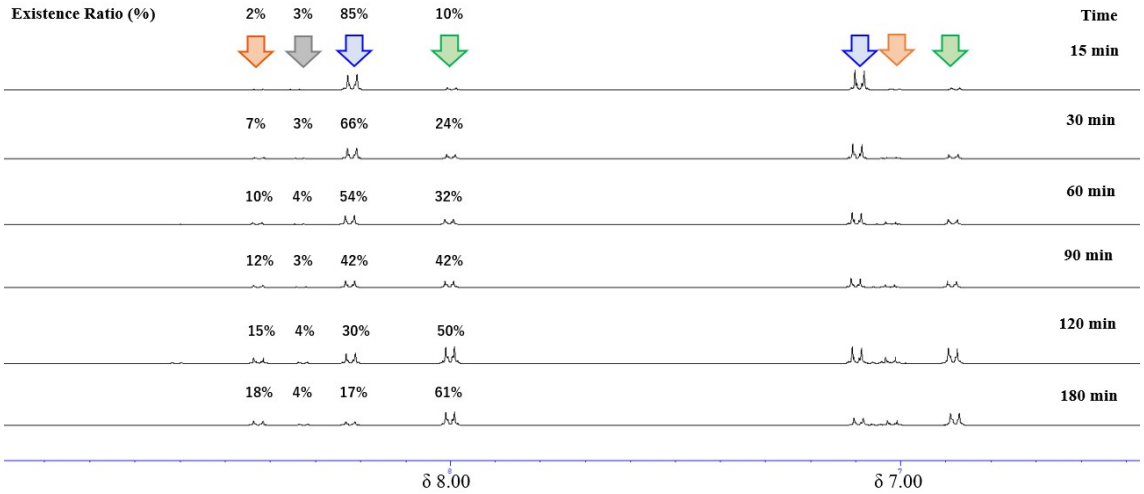


Figure S3 Enlarged NMR chart of stability of CDAP in D_2O. Normalized peak intensity of CDAP **6** (blue arrow), DMAP **11** (green arrow), compound **12** (orange arrow) and unidentified compounds (gray arrow) calculated from NMR spectra.

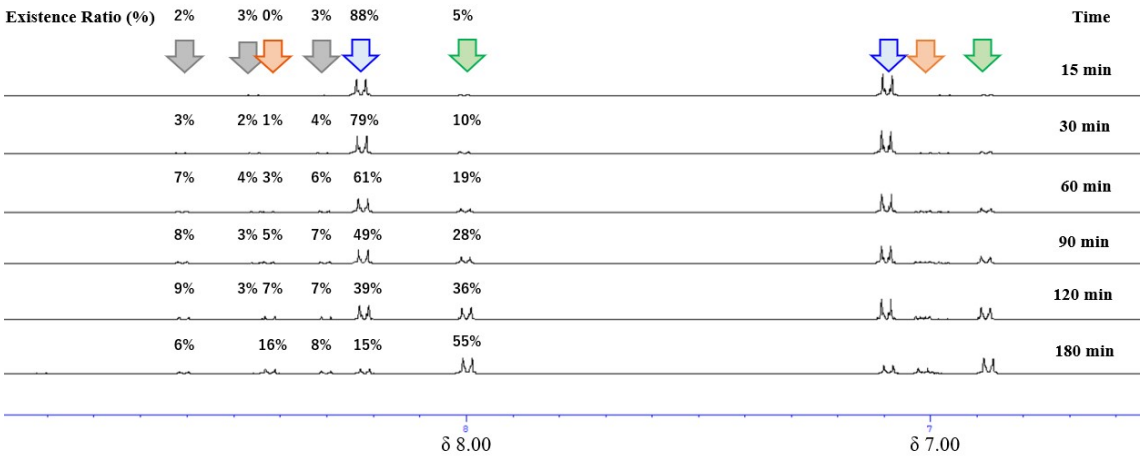
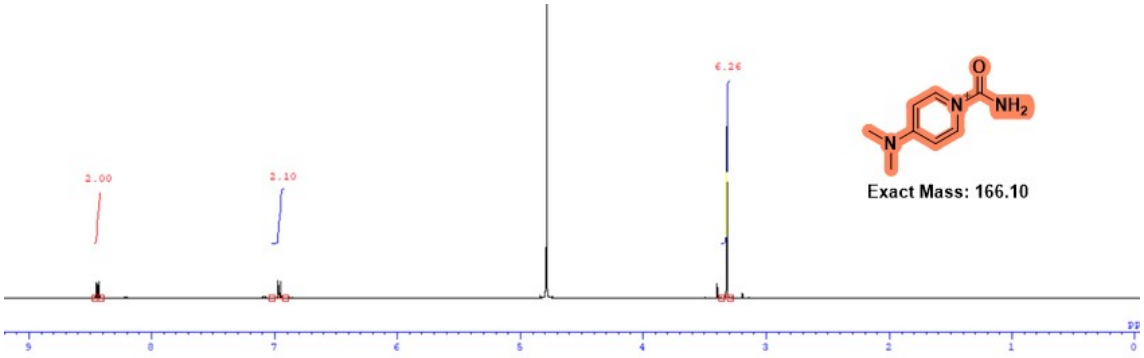


Figure S4 Enlarged NMR chart of stability of CDAP in D_2O with $ZnCl_2$. Normalized peak intensity of CDAP **6** (blue arrow), DMAP **11** (green arrow), compound **12** (orange arrow) and unidentified compounds (gray arrow) calculated from NMR spectra.

(A)



(B)

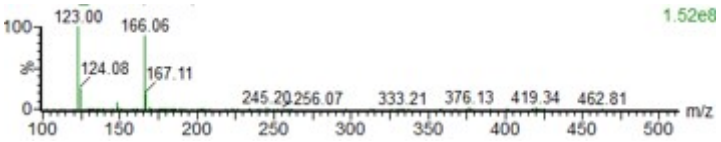


Figure S5 NMR chart and MS signal of compound **12.** (**A**)The structure of compound **12** and NMR analysis results after CDAP was added to D_2O containing hydrochloric acid and left to stand at room temperature for 17 h. (B)The MS signal in positive mode of compound **12**.

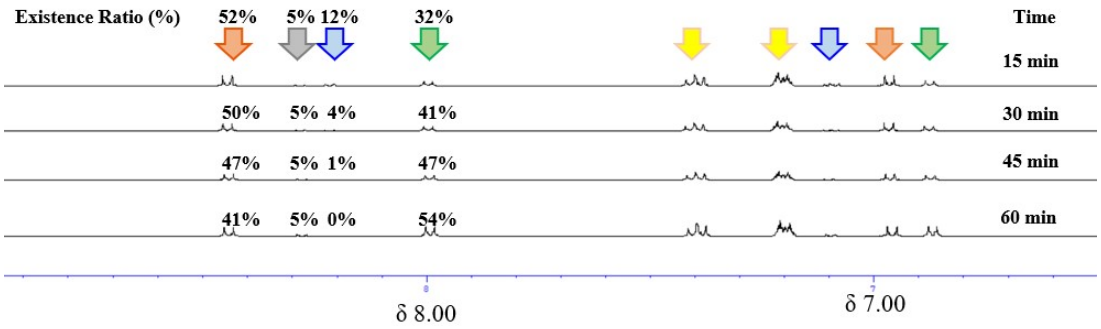
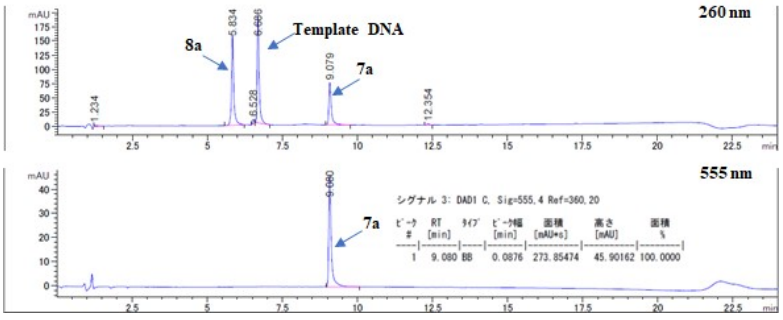
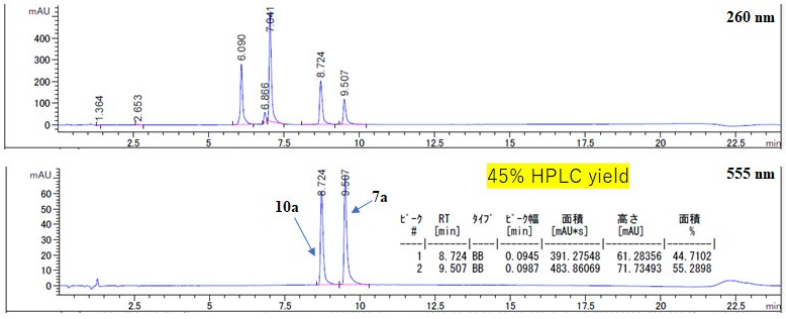
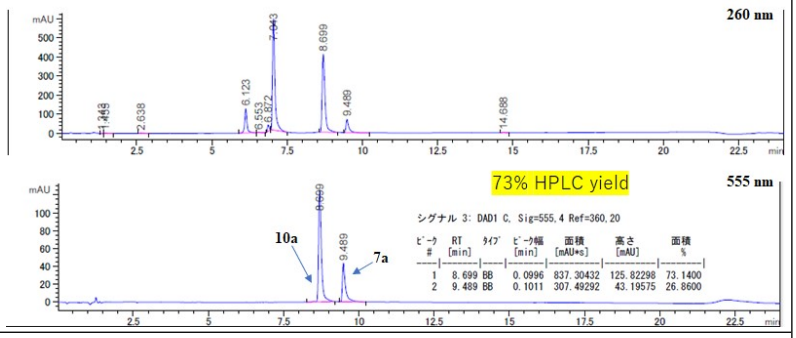
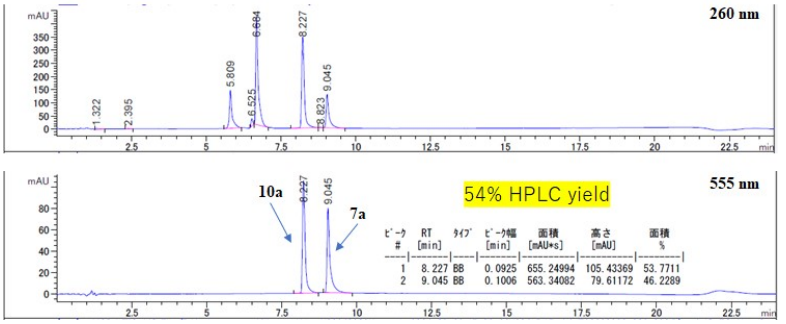
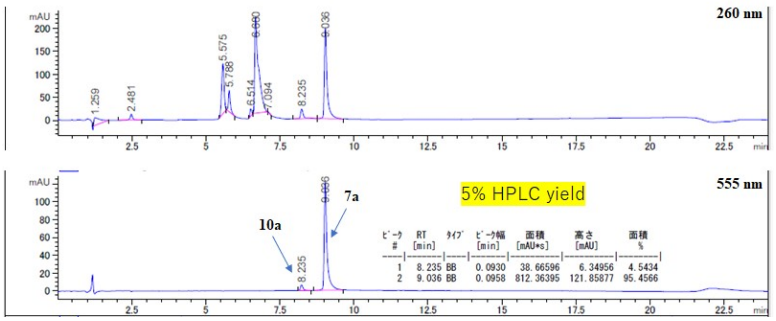
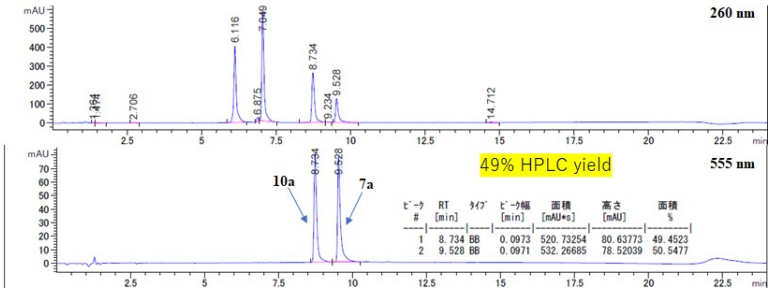
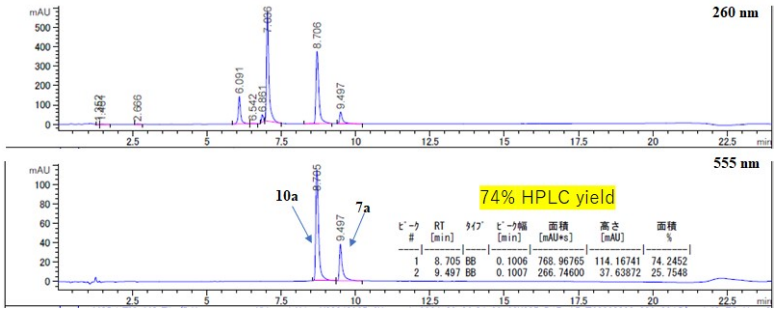
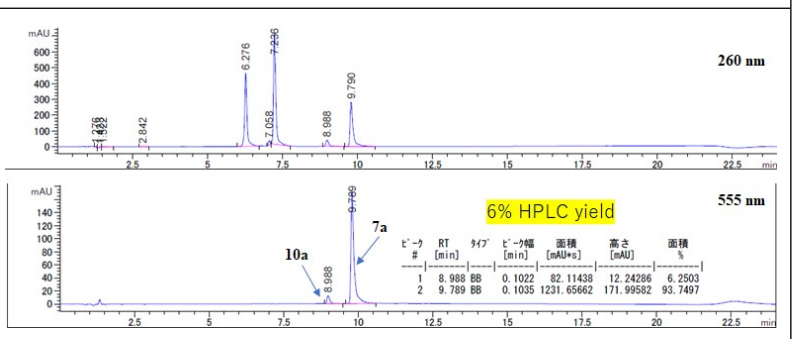
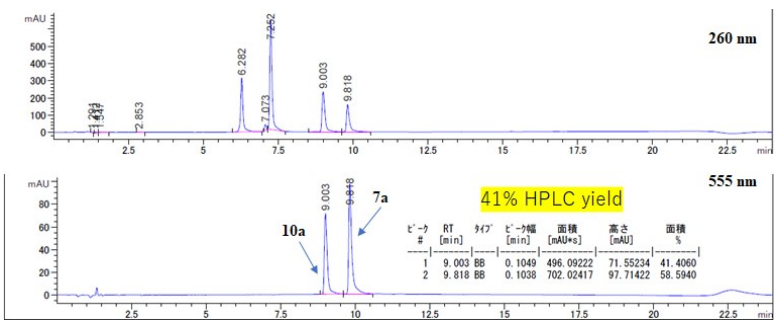


Figure S6 Enlarged NMR chart of Stability of CDAP in D_2O with phenyl phosphate and $ZnCl_2$. Normalized peak intensity of CDAP **6** (blue arrow), DMAP **11** (green arrow), compound **12** (orange arrow), phenyl phosphate (yellow arrow) and unidentified compounds (gray arrow) calculated from NMR spectra.

2. Supplementary Tables (Table S1–S3)

Table S1 Chromatograph of the chemical ligation using CDAP in Table 1.

Entry	Chromatogram
Starting materials	
1	
2	
3	

4	 260 nm 555 nm 5% HPLC yield 10a 7a
5	 260 nm 555 nm 49% HPLC yield 10a 7a
6	 260 nm 555 nm 74% HPLC yield 10a 7a
7	 260 nm 555 nm 6% HPLC yield 10a 7a
8	 260 nm 555 nm 41% HPLC yield 10a 7a

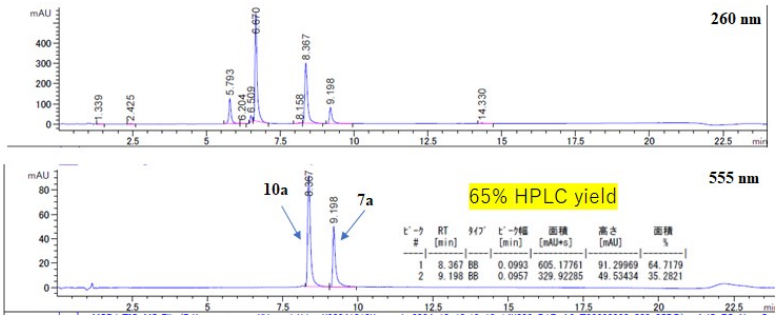
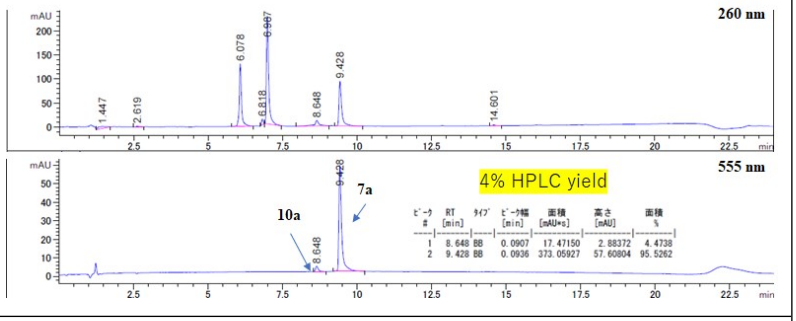
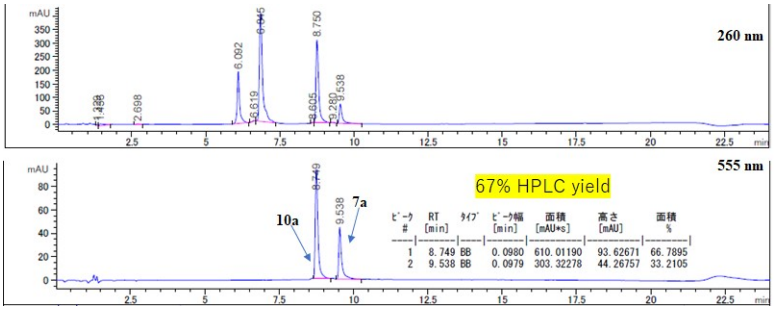
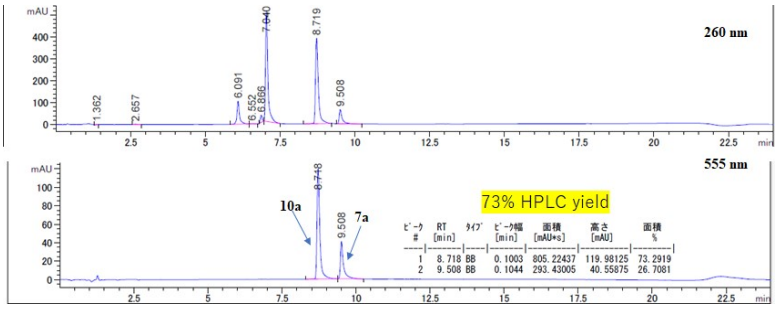
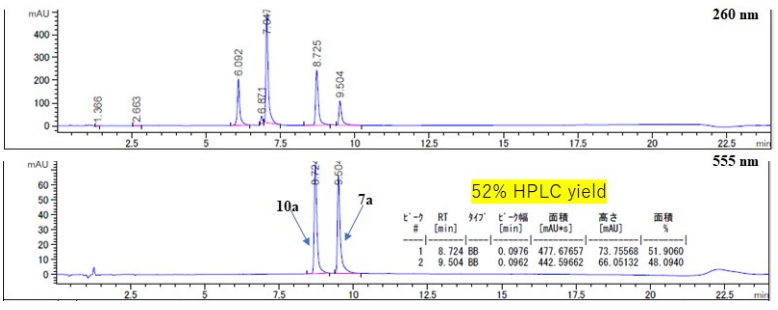
9	 260 nm 555 nm 10a 7a 65% HPLC yield
10	 260 nm 555 nm 10a 7a 4% HPLC yield
11	 260 nm 555 nm 10a 7a 67% HPLC yield
12	 260 nm 555 nm 10a 7a 73% HPLC yield
13	 260 nm 555 nm 10a 7a 52% HPLC yield

Table S2 Chromatograph of the chemical ligation using CDAP in Scheme 1

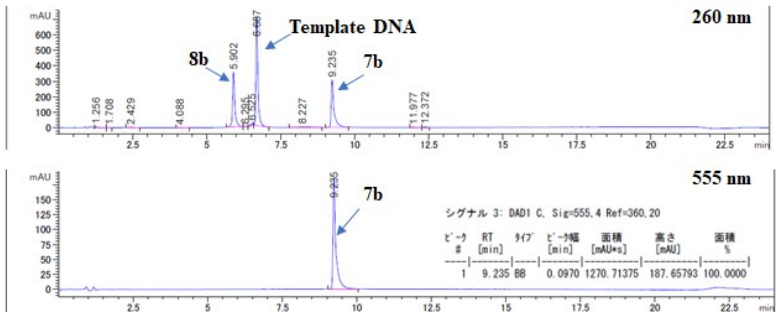
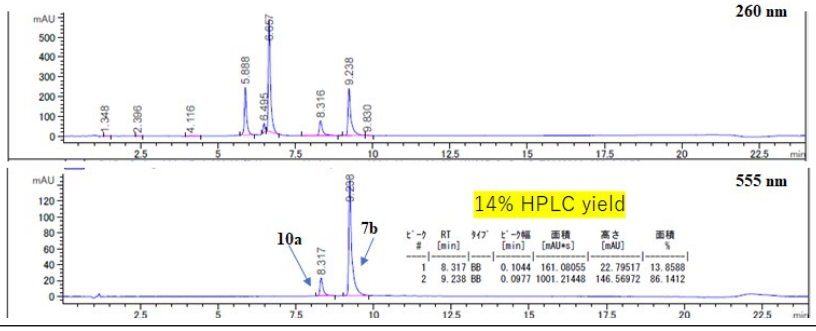
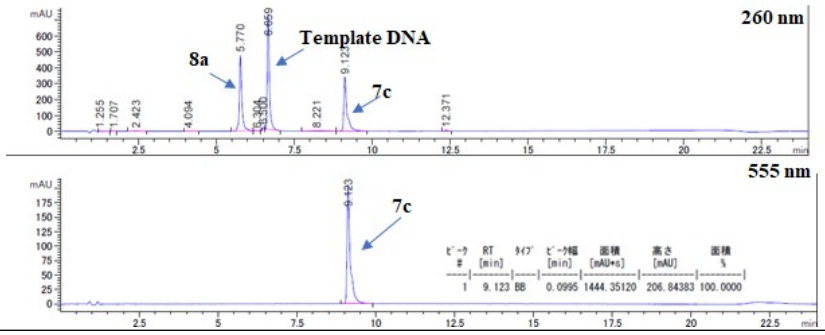
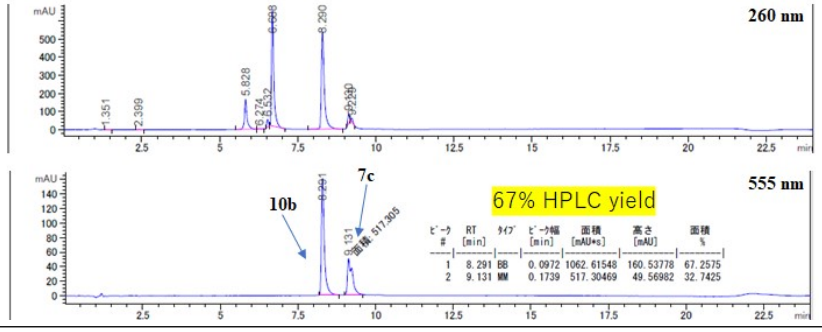
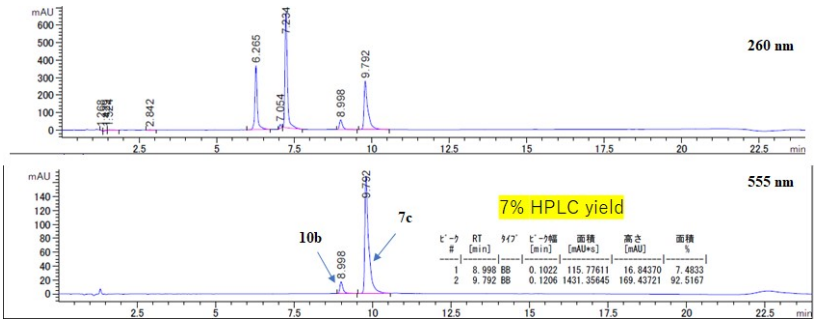
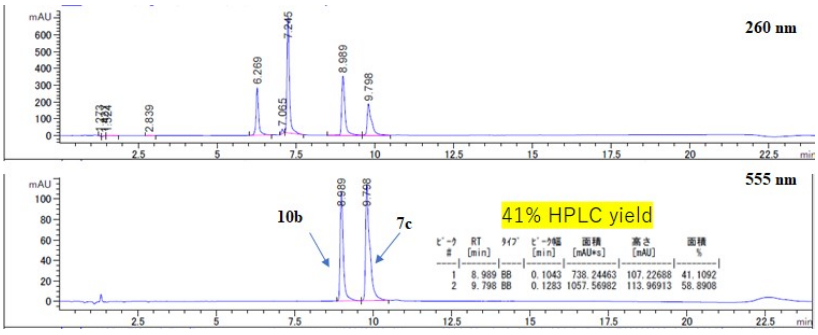
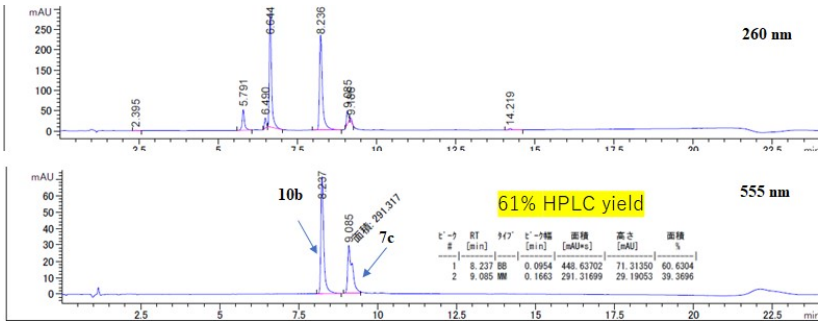
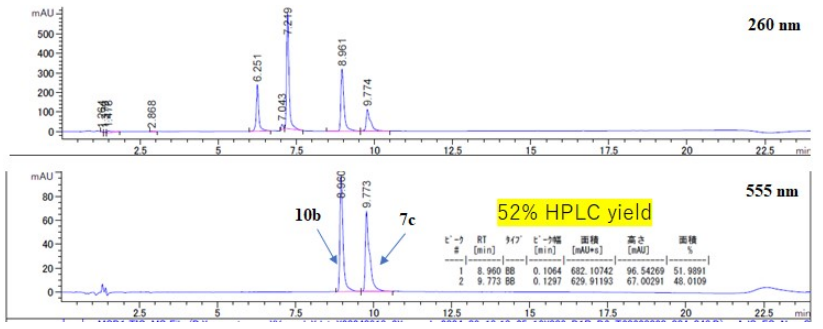
Sample	Chromatogram
Starting materials	
Reaction	

Table S3 Chromatograph of the chemical ligation using CDAP in Table 2.

Entry	Chromatogram
Starting materials	
1	
2	
3	

4	 260 nm 555 nm 10b 7c 61% HPLC yield
5	 260 nm 555 nm 10b 7c 52% HPLC yield

3. Supplementary methods

All DNA oligos were purchased from Sigma-Aldrich, where they were synthesized using standard phosphoramidite methods. The reagents used are detailed in the following protocol.

NMR analysis

^{1}H NMR spectra were measured using a Bruker AVIII-400 (400 MHz). The samples were prepared as 1.00 mL D_2O solutions of CDAP (TCI, cat. No. C2348), phenyl phosphate (TCI, cat. No. B0026), and $ZnCl_2$ (Sigma-Aldrich, cat. No. 39059-100ML-F) at 12.8 mM each. The pH was adjusted to pH 4.90–5.30 using a small amount of NaOH aqueous solution (Fujifilm Wako, cat. No. 192-02175) or to pH 1.35 using hydrochloric acid (Fujifilm Wako, cat. No. 083-01095).

LCMS analysis

The LCMS analysis of ligated oligonucleotides was carried out under the following conditions.

Equipment	Agilent InfinityLab LC/MSD XT, AJS
Column	ACQUITY PREMIER Oligonucleotide BEH C18, 2.1 mm×100 mm 1.7μm, 130 Å
Mobile phase A	8.6 mM TEA/100 mM HFIP in H_2O
Mobile phase B	MeOH
Gradient elution	B 10% to 90% in 18 min
Flow	0.300 mL/min
Injection volume	40.0 μL

The LCMS analysis of compound **12** was carried out under the following conditions.

Equipment	ACQUITY UPLC H-Class PLUS system, SQ detector 2
Column	ACQUITY PREMIER BEH C18, 2.1 mmx50 mm 1.7 μm, 130 Å
Mobile phase A	0.1% Formic acid aqueous solution
Mobile phase B	Acetonitrile
Gradient elution	B 10% to 90% in 8.5 min
Flow	0.800 ml/min
Injection volume	10 μL

General procedure for chemical ligation of DNA using CDAP

50.0 μL solution of 200 mmol/L NaCl (Thermo Fisher scientific, cat. No. AM9759) containing 2.00 μmol/L 5′ fragment **7a–c**, 2.60 μmol/L 3′ fragment **8a**,**b** and 2.60 μmol/L template DNA **9** was heated

at 90 °C for 3 min in a thermal cycler, and cooled to room temperature for 30 min. To the solution, 40.0 μL of 100 mmol/L MES buffer, 5.00 μL of 100 μmol/L divalent metal aqueous solution, and 5.00 μL of 400 μmol/L CDAP (TCI, cat. No. C2348) DMSO solution were added at ambient temperature. In the case of the reaction on ice, the mixture was left on ice for 15 min before adding CDAP. After the reaction, the crude solution was purified using Oligo Clean & Concentrator (Zymo Research, cat. No. D4061), and the eluate was analyzed using LCMS.

A 200 mM NaCl solution of nucleic acids (2.00 μM 5′ fragment **7a–c**, 2.60 μM 3′ fragment **8a**,**b** and 2.60 μM splint DNA **9**) were prepared initially in a total volume of 50.0 μL. The mixture was heated at 90℃ for 3 min and then incubated at ambient temperature for 30 min. To the mixture was added 40.0 μL of 100 mM MES (Thermo Fisher scientific, cat. No. J63341.AP) buffer or nuclease free water, 5.00 μL of 100 mM divalent metal aqueous solution and 5.00 μL of 400 mM CDAP (TCI, cat. No. C2348) solution in DMSO for starting chemical ligation. The reaction was incubated under ambient temperature. After ligation, the reaction mixture was purified using Oligo Clean & Concentrator (zymo research, cat No. D4061), and the eluate was analyzed using LCMS.