Fast and Efficient Synthesis of Zorro-LNA Type 3'-5'-5'-3' Oligonucleotide Conjugates via parallel in-situ stepwise conjugation.

O.I. Gissberg,[#a] M. Jezowska[#b], E. M. Zaghloul[a], N.I. Bungsu[a,c], R. Strömberg[b], C.I.E. Smith[a], K. E. Lundin*[a], and M. Honcharenko*[b]

Supplementary information

1. **Zorro constructs used in the study.**
2. **15% PAGE-UREA gel showing the migration of the different ONs.**
3. **Examples of HPLC analysis from different Zorro arms constructs: A) crude oligonucleotide (ON)-PATA conjugate, B) crude oligonucleotide (ON)-N3 conjugate.**
4. **Examples of HPLC analysis showing crude reaction mixtures of synthesis of Zorro Type 3'-5'-5'-3' oligonucleotide conjugates.**
5. **Mass Spectra and HPLC Chromatograms of purified Zorro Type 3'-5'-5'-3' oligonucleotide conjugates.**
6. **Representative gel illustrating the strand invasion assay.**

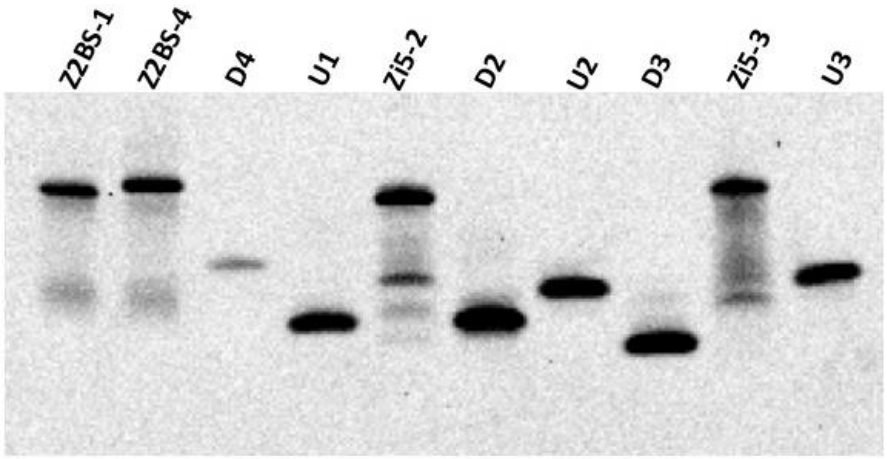
1. Zorro constructs used in the study

Zorro constructs used in the study

Name	overlap*	Sequence
Z-2ON	1	3′-AgTcgCgTAccCACgGTCtAaCt-5′ 5′-AGATtGACCCtCCtcTTtcTTCa3′
Z-2HEG	1	3′-AgTcgCgTAccCACgG-HEG-HEG-CCCtCCtcTTtcTTCa-3′
Z2BS-1	1	3′-AgTcgCgTAccCACgG click CCCtCCtcTTtcTTCa-3′
Z2BS-4	4	3′-AgTcgCgTAccCACgG click TGCcCCtCCtcTTtcTTCa-3′
Zi5-2	2	3′-GaAtcCctCcaAagG click CccCagCcaCccTctG-3′
Zi5-3	3	3′-CtcCggCacGaAcaAc click TtgCcAgaCtcTgCc-3′

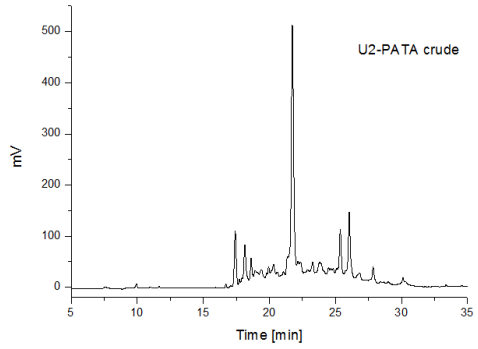
* bases overlapping between the two arms when the construct is bound to its dsDNA target

2. 15% PAGE-UREA gel showing the migration of the different ONs.

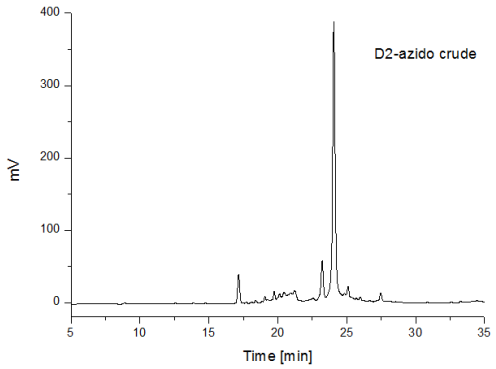


3. **Examples of HPLC analysis from different Zorro arms constructs:**

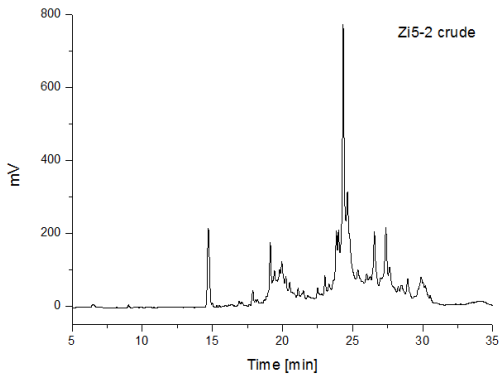
   A) **crude oligonucleotide (ON)-PATA conjugate,**

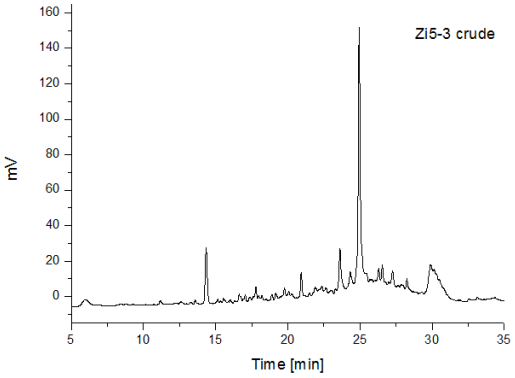


   B) **crude oligonucleotide (ON)-N3 conjugate.**



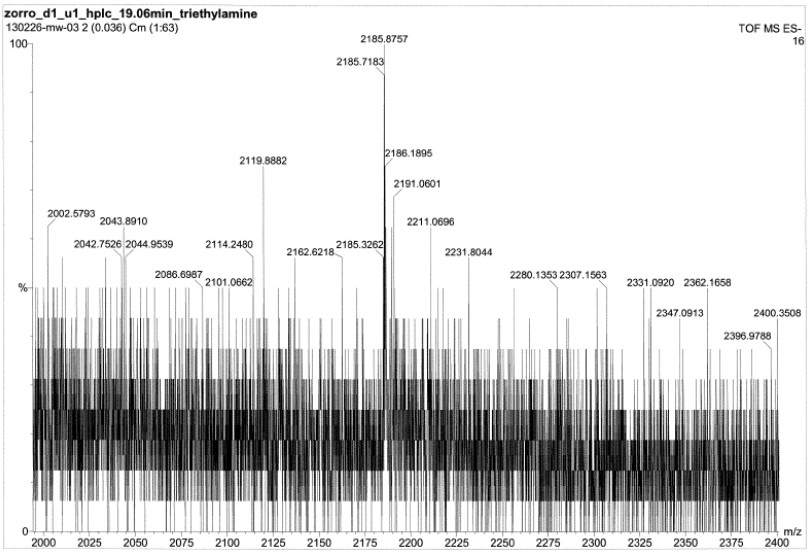
4. **Examples of HPLC analysis showing crude reaction mixtures of synthesis of Zorro Type 3'-5'-5'-3' oligonucleotide conjugates.**

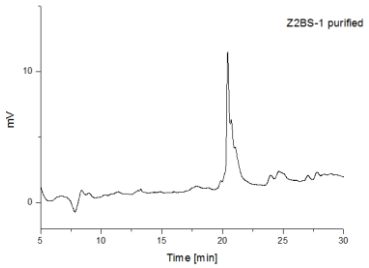




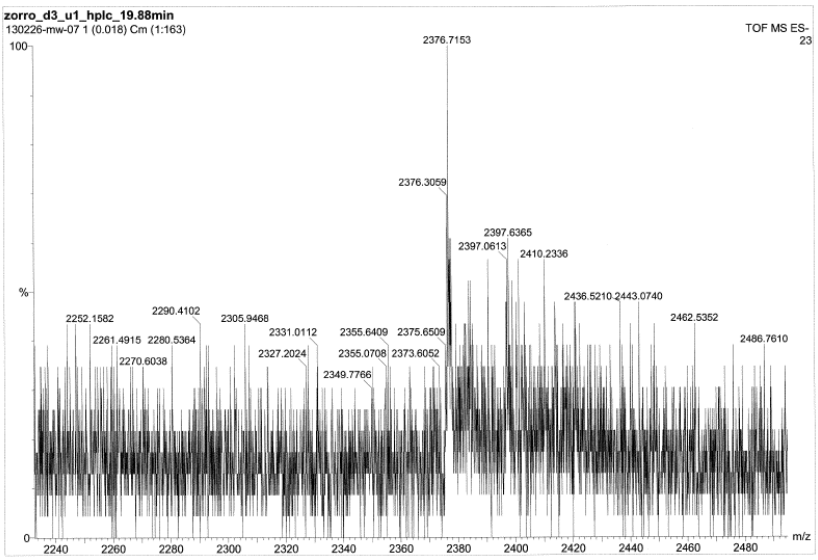
5. HPLC Chromatograms and Mass Spectra of purified Zorro Type 3’-5’-5’-3’ oligonucleotide conjugates.

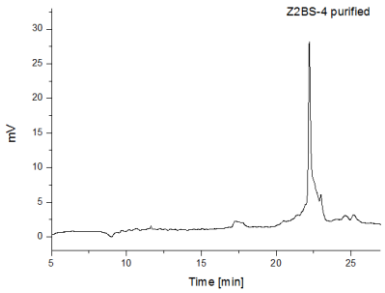
Z2BS-1



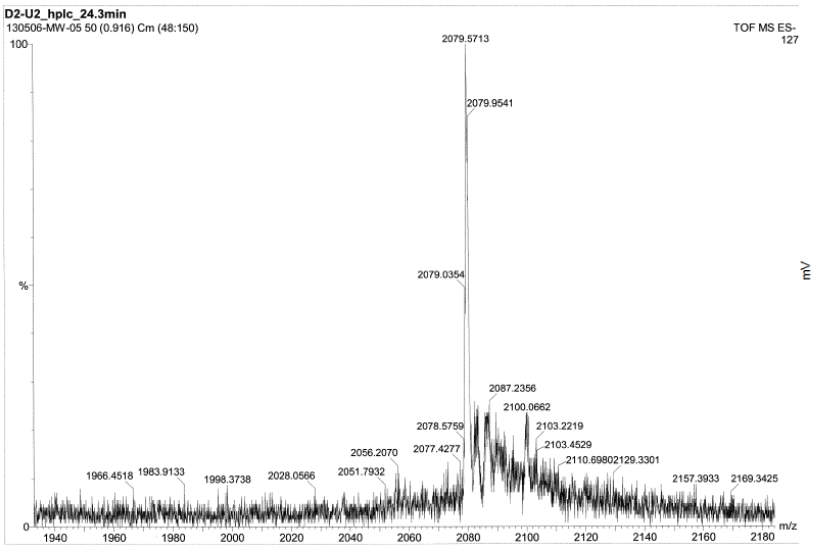


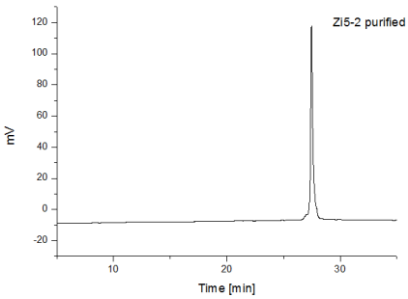
Z2BS-4



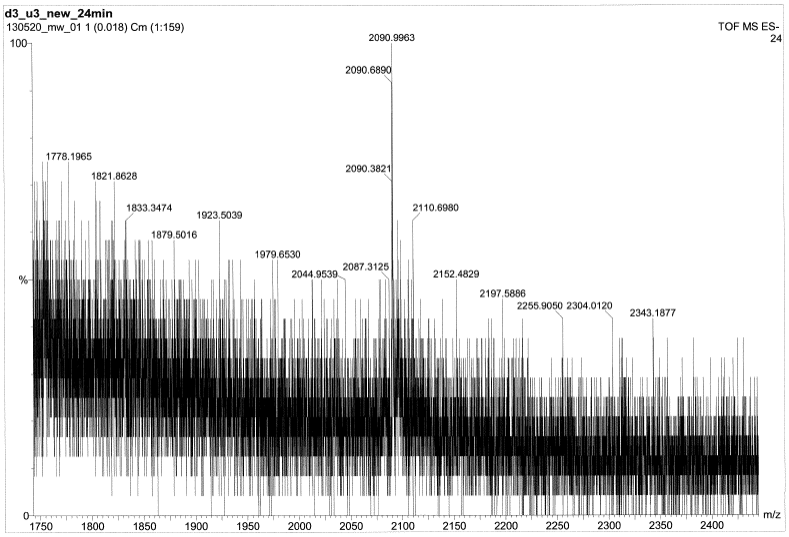


Zi5-2


D2-U2_hplc_24.3min
130506-MW-05 50 (0.916) Cm (48:150)
TOF MS ES-
127
2079.5713
2079.9541
2079.0354
2087.2356
2100.0662
2078.5759
2103.2219
2103.4529
2077.4277
2056.2070
2110.6980
2129.3301
2051.7932
1966.4518
1983.9133
1998.3738
2028.0566
2157.3933
2169.3425
%
m/z


Zi5-2 purified
mV
Time [min]

Zi5-3


d3_u3_new_24min
130520_mw_01 1 (0.018) Cm (1:159)
TOF MS ES-
24
2090.9963
2090.6890
2090.3821
1778.1965
1821.8628
1833.3474
1923.5039
1879.5016
2110.6980
1979.6530
2044.9539
2087.3125
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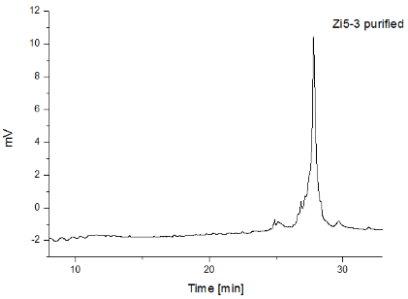
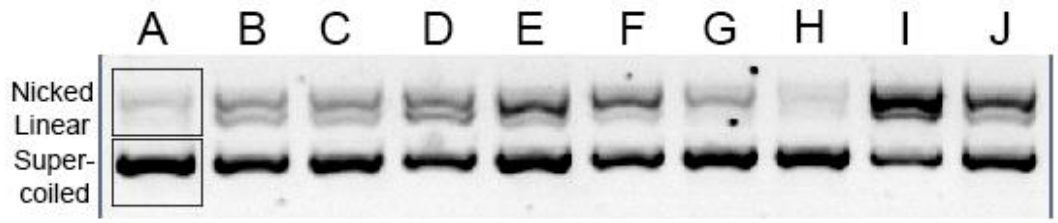
2197.5886
2255.9050
2304.0120
2343.1877
%
m/z


Zi5-3 purified
mV
Time [min]

6. Representative gel illustrating the strand invasion assay.



Plasmid together with ONs or Zorro constructs were incubated for 72 h at 37 °C and then treated with S1 nuclease and analyzed on a 1% agarose gel. The gel was stained with SYBR Gold and documented using the FluoroS gel documentation system (BioRad) equipped with a cooled CCD camera. The different plasmid fractions were quantified using the Quantity One software (BioRad). Lane **A** = plasmid pN25-2BS alone, **B** = Z2BS-4, **C** = Z2BS-1, **D** = U1 + D4, **E** = U1+D1, **F** = D4, **G** = D1, **H** = U1, **I** = Z-2HEG and **J** = Z-2ON. As a result of the strand invasion one DNA strand is displaced and creates a single stranded region in the plasmid. The S1 nuclease cleaves the single stranded DNA which results in increased bands corresponding to nicked and linear plasmid in relation to super coiled DNA. To calculate the strand invasion the value for the supercoiled band was divided by the total from the supercoiled + nicked + linearized bands and the ratio is then normalized to the ratio achieved for plasmid alone.