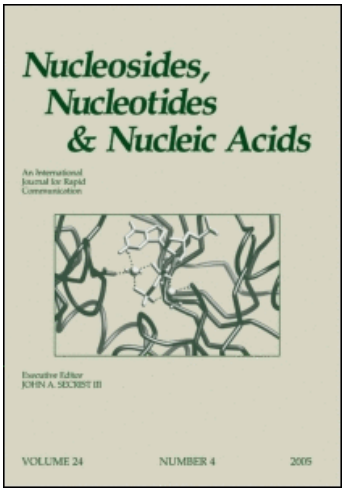

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

New Standards in LNA Synthesis

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Online publication date: 09 August 2003

To cite this Article Hansen, Henrik F. , Olsen, Otto and Koch, Troels(2003) 'New Standards in LNA Synthesis', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1273 — 1275
To link to this Article: DOI: 10.1081/NCN-120022944
URL: http://dx.doi.org/10.1081/NCN-120022944

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1273–1275, 2003

New Standards in LNA Synthesis

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Key Word: LNA phosphothioate synthesis (1-13 μmol).

LNA (locked nucleic acids) [1–3] has a close structural resemblance with DNA and uses standard DNA protecting schemes (DMT for the 5′-OH, Bz and *i*Bu for the nucleobases and CNE activation for the phosphate (Fig. 1). Therefore, previously reported preparations of LNA were performed using commercial DNA synthesizers and following essentially standard DNA procedures.

However, in our hands the preparation of LNA-oligos require modified procedures compared to standard DNA synthesis especially the coupling between LNA

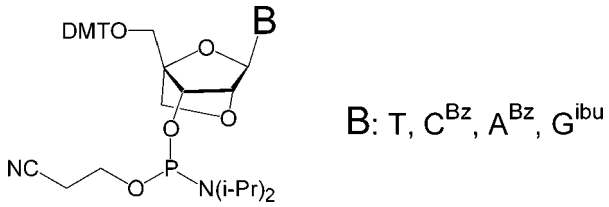

Figure 1. LNA-amedites monomers.

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DOI: 10.1081/NCN-120022944
1525-7770 (Print); 1532-2335 (Online)

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Table 1.

Scale	Eq monomer	Eq DCI pr monomer	Coupling time	Beaucage 10%	Cap (CV/min)	Yield purified
12 umol	4	8	LNA 15 min DNA 2.5 min	4 CV 300 cm/h	LNA 7.5/0.4 DNA 4/0.3	47%

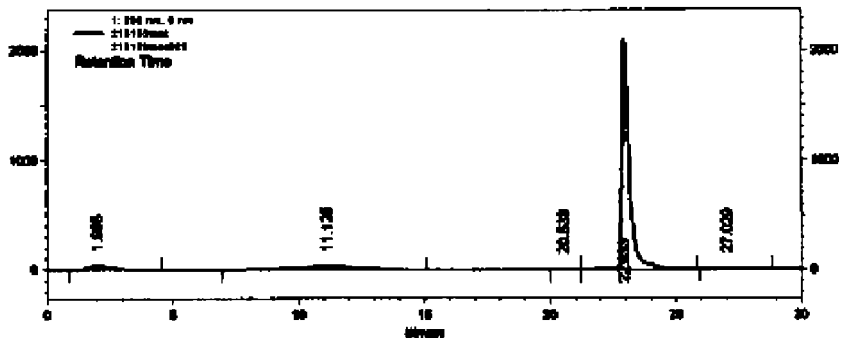

Figure 2. RP-HPLC 70 mg crude material DMT-on. Prep. collumn vydac 218TP101522 25×3 cm. Flowrate 40 mL/min. Buffer A: 0.1 m ammoniumacetate pH = 8, Buffer B: 100% acetonitrile. Gradient: 0 min 95% A : 5% B; 10 min 91% A : 9% B; 17 min 60% A : 40% B; 22–24 min 100% B; 25 min 95% A : 5% B.

Table 2.

Scale	Eq monomer 0.05 M	DCI Eq/monomer	Coupling time	Beaucage 4% (uL/min)	St. Ox (uL/s)	Cap (uL/s)
1 umol	8	9	435 s	480/3		480/45

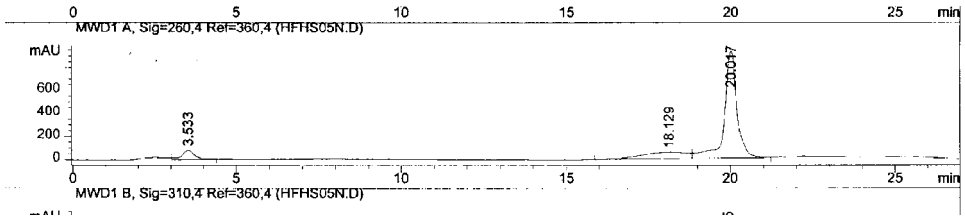

Figure 3. RP-HPLC crude material DMT-on. Collumn PLRR-S, 100 Å, 5 um. Flowrate 0.5 mL/min. Buffer A: 0.1 m TEAAc 10% acetonitrile, Buffer B: 0.1 M TEAAc 60% acetonitrile. Gradient: 0 min 85% A : 15% B; 15 min 60% A : 40% B; 20–22 min 100% B; 25 min 85% A : 15% B.

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and LNA requires prolonged couplings and oxidation times for both phosphorothioates and diesters. Table 1 lists the conditions for synthesis of a chimer LNA-DNA-LNA Dmt^{m}Cs^{m}CsGs-ts^{m}csas-ts^{m}cs^{m}Cs-^{m}Csts^{m}C-3′ on an Akta oligopilot 10 nucleic acid synthesis system and Fig. 2 show the HPLC of the crude oligo. Table 2 lists the conditions for synthesis of a 16 mer LNA phosphorothioate Dmt^{m}CsGsGs-As^{m}CsTs-AsAsGs-Ts^{m}Cs^{m}Cs-AsTsTs-Gs^{m}C-3′ on the Expedite nucleic acid synthesis system and Fig. 3 show the HPLC of the crude oligo.

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