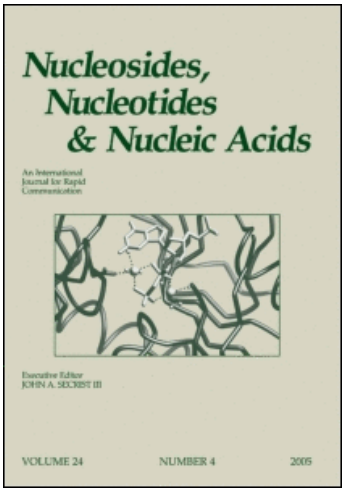

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[a] NOXXON Pharma AG, Berlin, Germany [b] Grünenthal GmbH, Aachen, Germany

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A New Class of Spiegelmers Containing 2′-Fluoro-nucleotides

S. Helmling,[1] E. Moyroud,[1,*] W. Schroeder,[1] I. Roehl,[1] F. Kleinjung,[1] S. Stark,[1] G. Bahrenberg,[2] C. Gillen,[2] S. Klussmann,[1] and S. Vonhoff[1]

[1]NOXXON Pharma AG, Berlin, Germany
[2]Grünenthal GmbH, Aachen, Germany

ABSTRACT

Synthesis of 2′-fluoro-nucleosides from L-arabinose in order to perform the synthesis of 2′-fluoro-Spiegelmers binding to a neuropeptide.

Key Words: 2′-Fluoro-Spiegelmer; 2′-Fluoro-nucleosides; Fluorination using DAST.

Spiegelmers are metabolically stable enantiomeric nucleic acid ligands with high affinity and specificity for a given target. Employing a modified SELEX process (Systematic Evolution of Ligands by Exponential enrichment) with a neuropeptide target consisting of D-amino acids and modified nucleoside triphosphates (2′-fluoro-dUTP and 2′-fluoro-dCTP), we identified 6 different sequences containing 2′-fluoro-dU and 2′-fluoro-dC that specifically bind the enantiomeric target. The clone with the best binding activity was further optimized and truncated. In order to bind the natural peptide (a neuropeptide), the mirror image of this molecule (2′-fluoro-Spiegelmer) was synthesised.

*Correspondence: E. Moyroud, NOXXON Pharma AG, Gustav Meyer Allee 25, 13355, Berlin, Germany; Fax: +49 304 630 7225; E-mail: emoyroud@noxxon.net.

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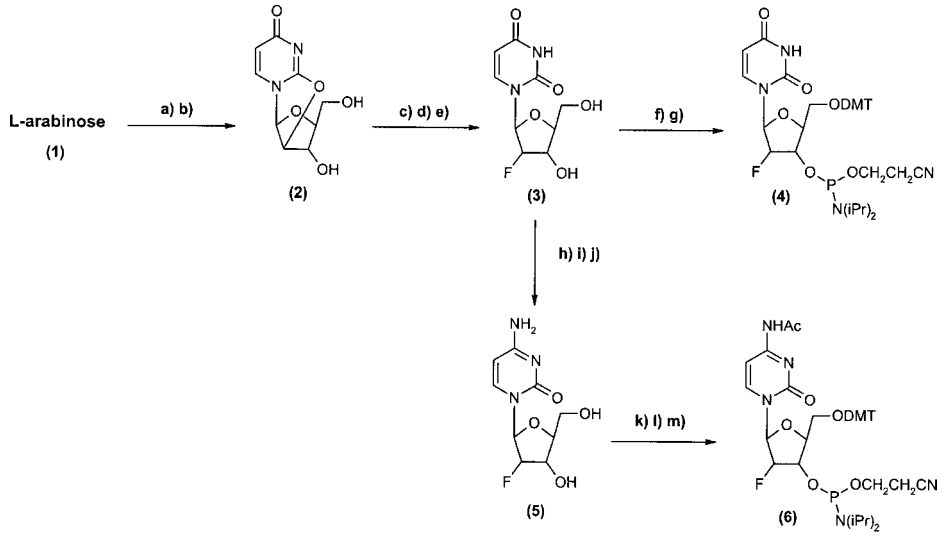

Scheme 1. Synthesis of 2′-fluoro-L-uridine and 2′-fluoro-L-cytidine phosphoramidites (**4**) and (**6**): a) Cyanamide, MeOH; b) methylpropiolate, EtOHaq; c) PxCl, Pyr; NaOH, MeOH; d) DAST, NaF, TEA, ACN, DMF; e) HCl, MeOH; f) DMTCl, DMAP, Pyr; g) $Cl(OCH_2CH_2)P(N(iPr)_2)$, DIPEA, DMAP, THF; h) BzCl, Pyr; i) 4-chlorophenyldichlorophosphate, 1,2,4-triazol, Pyr; NH_3 (28% aq), dioxane; j) $MeOH/NH_3$ sat.; k) Ac_2O, DMF; l) DMTCl, DMAP, Pyr; m) $Cl(OCH_2CH_2)P(N(iPr)_2)$, DIPEA, DMAP, THF.

For the chemical synthesis of 2′-fluoro-Spiegelmers, we prepared 2′-fluoro-L-uridine- and 2′-fluoro-L-cytidine phosphoramidites ((**4**) and (**6**)) starting from L-arabinose (**1**) (Sch. 1).

2,2′-Anhydro-L-uridine (**2**) was synthesised from L-arabinose (**1**) according to literature.[1] After protection of the hydroxyl groups with pixyl chloride (PxCl) and treatment with NaOH, the crude mixture was fluorinated using DAST.[2] To increase the concentration of fluoride in the mixture NaF was used in large excess. After treatment with HCl (1N) and neutralisation, the crude compound **3** was tritylated and phosphitylated to give the 2′-fluoro-L-uridine phosphoramidite (**4**). The 5 step-synthesis from compound **2** to 5′-DMT-2′-F-U was done at large scale (30–50 g) without purification of the intermediates in 20–30% overall yield. For the synthesis of 2′-fluoro-L-cytidine phosphoramidite (**6**), 2′-fluoro-L-uridine (**3**) was converted in 3 steps to 2′-fluoro-L-cytidine (**5**) in 50–70% overall yield (reaction scale: 30–50 g, without purification of the intermediates) using the 1,2,4-triazole chemistry.[3–5] The crystalline compound **5** was selectively acetylated, tritylated and phosphitylated to give compound **6**.

2′-Fluoro-Spiegelmers were synthesised DMT-on, on an Äkta synthesizer (15–20 μmol scale), using a concentration of 0.05 M phosphoramidites and coupling times of 20 min for the 2′-fluoro-L-phosphoramidites and 10 min for the L-phosphoramidites. The 2′-fluoro-L-RNA molecules were purified using RP-HPLC, detritylated and desalted (Fig. 1).

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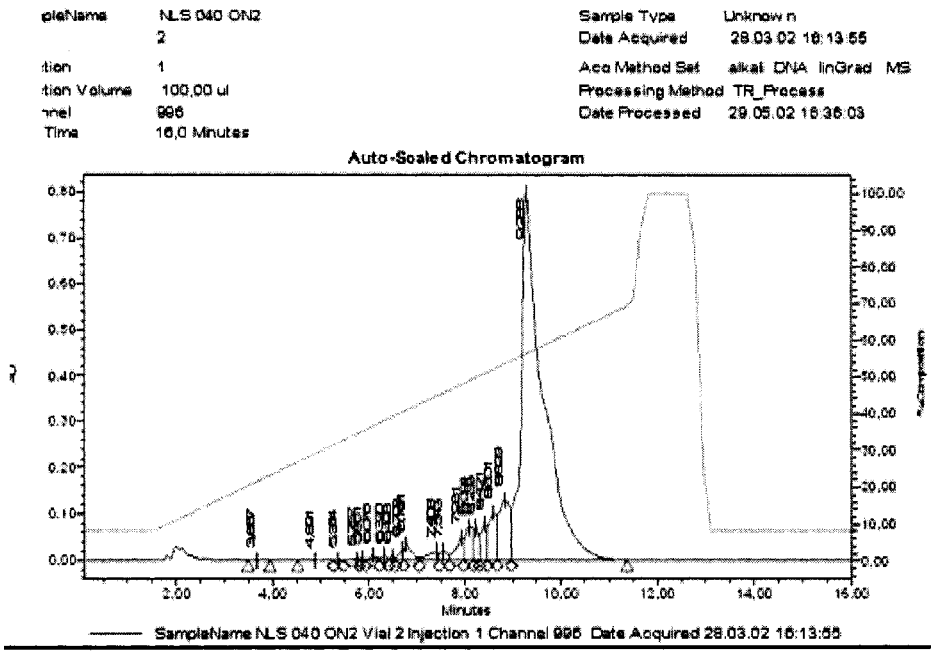

Figure 1. IX-HPLC chromatogram of the 59-mer 2′-fluoro-Spiegelmer under strong denaturing conditions. (Eluent A: 10 mM NaOH; 1 mM EDTA; 10 mM $NaClO_4$ in 10% ACN, Eluent B: 500 mM $NaClO_4$ in A).

CONCLUSIONS

1. The synthesis of 2′-fluoro-L-uridine and 2′-fluoro-L-cytidine phosphoramidites ((**4**) and (**6**)) were performed (without purification of the intermediates at 30–50 g scale).
2. The synthesis of Spiegelmers containing 2′-fluoro-nucleotides was achieved.
3. High affinity 2′-fluoro-Spiegelmers that bind a neuropeptide were identified.
4. The full-length ligand was truncated to 55 nt without loss of binding.
5. The affinity of the Spiegelmer to the neuropeptide was evaluated by measuring the binding constant on ITC (Isothermal Titration Calorimetry).
6. Moreover, in a cell culture assay was demonstrated the strong binding of 2′-fluoro-Spiegelmer to the neuropeptide and the inhibition of receptor activation.

ACKNOWLEDGMENT

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