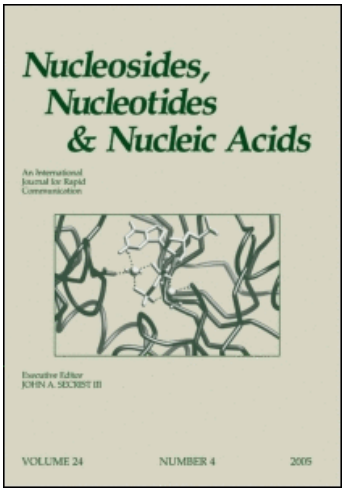

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Peptide-and Biotin-Oligonucleotide-Pyrrole Conjugates for Electrochemical Addressing on Silicon Chip

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PEPTIDE- AND BIOTIN- OLIGONUCLEOTIDE-PYRROLE CONJUGATES FOR ELECTROCHEMICAL ADDRESSING ON SILICON CHIP

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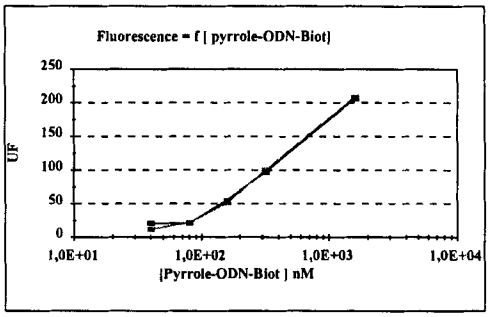
ABSTRACT: The syntheses of pyrrole-oligonucleotide-peptide conjugates and pyrrole-oligonucleotide-biotin conjugates were described. The oligonucleotide moiety acted as an « active linker» which allowed the easy purification and quantitation of the conjugates and in turn controlled the grafting. The peptide conjugates were immobilised on a silicon array and their immunoreactivity was tested using biotinylated antibodies and a phycoerythrin-streptavidin staining. The biotin conjugate provided a fluorescence scale.

Pyrrole-ODN conjugates: Pyrrole-propionic acid N-hydroxysuccinimide ester was reacted with 4-N-(6-aminohexyl)-5-methyl-5'-O-DMT-2'-deoxycytidine[1] **1** and the resulting intermediate **2** (86% yield) was converted to the phosphoramidite **3** (80% purified yield) R_f=0.24 & 0.35 in CH_2Cl_2/AcOEt/TEA(50:45:5). The ^{1}H-NMR (acetone-d_6) displayed characteristic signals at 6,8 (α-pyrrole) and 6,06ppm (β-pyrrole). For the ODN synthesis, either a 4-N-(6-biotinamidohexyl)- or a 4-N-(6-trifluoramidohexyl)-5-methyl-2'-deoxycytidine unit[1] was introduced at the 3'end, after synthesis of a poly-T chain, the pyrrole-phosphoramidite **3** (0.1M in CH_3CN) was coupled at the 5' position using the trityl-off option. After standard ammonia deprotection, the resulting $^{5'}$(^{Pyr}dC-T_{13}-dC^{bio}) **4** was isolated by HPLC[2] (Rt=25mn), the lipophilic character of the pyrrole residue allowed an easy separation from the failed sequences (Rt~18.5mn). UV(H_2O): λ_{max} = 265 nm (ε_{265}~ 130 000 taking ε_{265} = 9 100 for ^{Pyr}dC and ^{bio}dC, 8 400 for T). MS (ES^-): M-H^- = 5043.8 (calc. : 5044.79). Similarly the aminohexyl-ODN $^{5'}$(^{Pyr}dC-T_{11}-dC^{AH}) **5** was isolated (Rt= 23.6 mn)[2]. MS (ES^-): M-H^- = 4209.4 (calc. : 4210.1).

Pyrrole-ODN-peptides conjugates: The aminohexyl-ODN **5** (24 nmol) in 12 µl of MOPS (50mM, pH 7.6) was treated with disuccinimidyl suberate (2.5 µmol in 25µl DMF) overnight at 4°C, desalted (NAP 10) and concentrated by n-Butanol extraction. The unmodified ACTH fragment 11-24 (65 nmol in 100µl MOPS) was added to the ODN. The pyrrole-ODN-ACTH(11-24) conjugate **6** was isolated (Rt = 26.4 mn)[3]. MS (ES^-) : $M\text{-}H^-$ = 5999.4 (calc. 6000.33). Similarly an ACTH(18-39) conjugate **7** was made.

Addressing of Pyrrole-ODN conjugates on silicon chip: The conjugate **6** and **7** (1.4µM in 20 mM pyrrole), were electropolymerised on electrodes 5/7 and 4/6/8/9 respectively of a 48 x 50µm^2 electrodes silicon chip as described earlier[4]. The chip was incubated either with biotinyl-MAB(18-24) or biotinyl-MAB(34-39) (10µg/ml PBS, RT, 45mn) and then with SA-PE[4]. Epifluorescence[4] showed that MAB(18-24) gave positive signal on dots 4 to 9 and MAB(34-39) gave positive signals only on dots 5/7 as expected. Conjugate **5** was electropolymerised as above (duplicates) using the respective concentrations 1.6µM, 320, 65, 32 and 16 nM. After incubation with SA-PE, fluorescence intensities measured on spots correlated well to the initial conjugates concentration (See graph).



CONCLUSIONS: These results show that the conjugation of biomolecules to a pyrrole-ODN scaffold, allow simple gel exclusion purification (increased M .W), HPLC purification at neutral pH and easy quantitation by UV absorption. Electropolymerized . peptides conjugates retain their immunoreactivity. Control of the pyrrole/biotin-conjugate ratio during the polymerization give rise to a fluorescent scale on the chip (quantitation).

REFERENCES

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2. RP-18E (10µm) 250X10 ; 10% to 16% CH_3CN in 50mM TEAA in 20mn, 5ml/mn.

3. RP-18E (5µm)125X4 ;10% to 18% CH_3CN in 20mn, then to 27%in 15mn, 1ml/mn.

4. Livache T., Fouque B., Roget A., Marchand J., Bidan G., Teoule R . and Mathis G. *Anal. Biochem.*,**1998** , *255* , 188-194.