ELSEVIER

Journal of Chromatography A, 764 (1997) 95–102

JOURNAL OF CHROMATOGRAPHY A

Ketone electrophores and an olefin-release group electrophore-labeled DNA oligomer Detection via electron capture

Linxiao Xu[1], Daniel Magiera, Helena Abushamaa , S. Kugabalasooriar, Roger W. Giese*

Department of Pharmaceutical Sciences in the Bouvé College of Pharmacy and Health Professions, Barnett Institute, and Chemistry Department, Northeastern University, Boston, MA 02115, USA

Received 27 August 1996; accepted 22 October 1996

Abstract

Sixteen ketone electrophores were synthesized (4′-pentafluorobenzyloxyacetophenone, **1**, and fifteen analogs thereof). As intended, each gave a high response by gas chromatography with electron capture detection (GC–ECD) as well as GC with detection by electron capture mass spectrometry (GC–EC-MS). Each spectrum by the latter technique consisted of a single major ion. As a representative compound, **1** was converted to a corresponding olefin, N-hydroxysuccinimide ester, which, in turn, was attached covalently to a DNA oligomer. Detection of the latter species spotted onto a nylon membrane was achieved by the sequence permanganate oxidation (which reformed and thereby released **1**), hexane extraction, silica solid phase extraction and GC–EC-MS (giving a peak for **1**). These results demonstrate potential for olefin-release group electrophores to function as multiplicity labels in assays involving DNA probes.

Keywords: Mass spectrometry; Ketone electrophores; DNA oligomer; 4′-Pentafluorobenzyloxyacetophenone

1. Introduction

DNA probes are widely employed as detection reagents. Such reagents contain a label such as a radioisotope, making it convenient to monitor the hybridization of the probe onto a target DNA, where the latter contains a complementary sequence for the probe.

Increasingly, labels other than radioisotopes are being employed for DNA probes, as has been reviewed in Ref. [1]. These include fluorophores, chemilumiphores and enzymes. Motivation for this comes not only from the negative properties of radioisotopes, such as safety and cost, but also from the positive properties of some non-isotopic labels. For example, multiplicity has been achieved with fluorophore-labeled DNA probes [2]. To achieve this, different DNA probes are labeled with different fluorophores, or a combination of fluorophores, and the fluorophores are selected so that the probes can be distinguished even when combined in a single experiment. However, only low-to-moderate multiplicity has been demonstrated so far with fluorophore–DNA probes. This is because the chemical and physical properties of fluorophores are not well-

*Corresponding author. Address for correspondence: Chemistry Department, Northeastern University, Boston, MA 02115, USA.
[1] Current address: Research Biochemicals International, Natick, MA, USA.

0021-9673/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved
PII S0021-9673(96)00915-6

suited for high multiplicity. DNA sequences as labels provide high multiplicity [3], but can be tedious to detect.

We are pursuing electrophores as labels for DNA probes. Electrophores are compounds that are detected in the gas phase by a method involving electron capture. Usually, this entails gas chromatography with electron capture detection (GC–ECD), or GC with detection by electron capture mass spectrometry (GC–EC-MS). Primarily, the potential of electrophores for high multiplicity makes them of interest as labels for DNA probes [4], but their high sensitivity also is important. Our approach to employing electrophores in this way is to prepare DNA probes in which the electrophore is attached covalently to the DNA via a suitable 'release group'. For detection, this latter group is cleaved, releasing the electrophore in a volatilizable form for measurement via electron capture.

In this paper, we report the preparation and electron capture detection properties of a number of ketone electrophores. Also, we demonstrate, with one of the electrophores, a general method for attaching such compounds, via an olefin-release group, to a DNA oligomer. Finally, the detection of the resulting electrophore-labeled DNA probe spotted onto a nylon membrane is shown.

Our prior work in this area has included studies of methionylamide [5] and trichloroacetamide (and related) thermal release groups [6]. Also, we have studied a model electrophore involving an olefin release group [7]. Others have used photolytic and catechol diether release groups to demonstrate that electrophores can be used as multiplicity labels to monitor combinatorial synthesis on beads [8]. Oligonucleotides labeled with a nonreleasable electrophore have been detected by matrix-assisted laser desorption/ionisation mass spectrometry [9].

2. Experimental

2.1. Chemicals and materials

A 5′-amino link modified DNA 20-mer was purchased from Oligo's Etc. (Wilsonville, OR, USA). Sodium bicarbonate was purchased from Life Technology (Grand Island, NY, USA). DNA bases were from Sigma (St. Louis, MO, USA). Ammonium hydroxide (30%), formic acid (88%) and potassium permanganate were from Fisher Scientific (Boston, MA, USA). N-Hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), sodium hydroxide and HPLC-grade glacial acetic acid were from Aldrich (Milwaukee, WI, USA). Distilled–deionized water was obtained with a Nanopure/Organic pure system (Barnstead, Boston, MA, USA). Ultra high purity helium, ultra high purity nitrogen, C.P.-grade methane (99.998%) and Oxisorb-LP cartridges were obtained from MedTech Gases (Medford, MA, USA). Dioxane, lithium aluminum hydride, reagent-grade triethylamine (TEA), deuterated acetone, HPLC-grade acetonitrile, hexanes, methylene chloride, methanol and ethyl acetate solvents were purchased from Baker (Phillipsburgh, NJ, USA). Charged nylon hybridization transfer membrane was obtained from MSI (Westbaro, MA, USA).

2.2. Equipment

The HPLC system consisted of a Series-4 liquid chromatograph from Perkin-Elmer (Norwalk, CT, USA), a Rheodyne Model 7125 injector from Rainin (Woburn, MA, USA) and a Spec-100 UV detector (260 nm) from Spectraphysics (Piscataway, NJ, USA). All solvents were filtered through 0.22-μm nylon filters obtained from MSI (Haneoye Falls, NY, USA), degassed under vacuum and purged in the solvent cabinet under 5–7 p.s.i. of helium (1 p.s.i.= 6894.76 Pa).

GC with on-column injection was performed with a 5890 GC from Hewlett-Packard (Palo Alto, CA, USA) equipped with a ^{63}Ni electron capture detector and an Ultra II HP capillary column (12.5 m×0.54 mm I.D., 0.33 μm film thickness) from Hewlett-Packard. The flow-rate of the carrier gas (He) was set to 1.0 ml/min and that of the make-up gas (N_2) to 44 ml/min. The following column program was used: Inject at 150°C and hold for 5 min, then ramp to 260°C at 10°C/min. The injector was started at 50°C and immediately programmed at 80°C/min to 260°C. The electron capture detector temperature was 350°C.

GC–EC-MS was performed with a Hewlett-Packard 5988A mass spectrometer coupled to a HP 5890 gas chromatograph. The mass spectrometer–gas

chromatograph was connected to a HP 59970C (Rev. 3.2) MS Chemstation computer for data acquisition and processing by single ion monitoring (SIM). The GC–MS of a released electrophore was done on an Ultra II HP capillary column (25 m×0.32 mm I.D., 0.17 μm film thickness) from Hewlett-Packard. The carrier gas (helium) was set to 20 p.s.i. column head pressure and methane was used at a source pressure of 2.0 Torr (250°C) (1 Torr=133.322 Pa). The following GC column program was run to determine the released electrophore: Inject on-column at 200°C, hold for 2 min at 50°C initial column temperature, ramp column oven to 300°C at 50°C/min and hold for 5 min.

2.3. Synthesis

2.3.1. 4′-pentafluorobenzyloxyacetophenone, 1

A mixture of 4-hydroxyacetophenone (5 mmol), pentafluorobenzyl bromide (1.3 g, 5 mmol), potassium carbonate (5 g), acetone (30 ml) and 18-crown-6-ether (0.1 g) were stirred under reflux for 5 h. After addition of some Celite 521, the reaction mixture was filtered and the residue was washed three times with acetone. Then the filtrate was evaporated on a rotary evaporator (rotavapor). The product was purified by recrystallization (hexane–ethyl acetate). Yield: 90%. m.p. 88–89°C. ^{1}H NMR, δ: 2.56 (s, 3H, CH_3), 5.20 (s, 2H, CH_2O), 7.02 (δ, 2H, J=8.8 Hz, H-C3′ and H-C5′), 7.96 (d, 2H, J=8.8 Hz, H-C2′ and H-C6′) ppm; ^{13}C NMR δ: 26.1 (CH_3) 57.3 (CH_2O) 109.5 (C1′, $^2J_{C\text{-}F}$=18 Hz), 114.2 (C3′ and C5′), 130.5 (C2′ and C6′), 131.1 (C1′), 137.5 (C2″ and C6″, $J_{C\text{-}F}$=248 Hz), 141.8 (C4″, $J_{C\text{-}F}$=256 Hz), 145.6 (C3″ and C5″, $J_{C\text{-}F}$=251 Hz) 161.5 (C4′) 196.4 (C=O) ppm. MS (EI): 316 (M^+); ECD-MS: 135([M−PFBz]$^-$, 100%).

2.3.2. Methyl 3-[4′-(pentafluorobenzyloxy)-phenyl]crotonate, 1a

The reaction was carried out under nitrogen. To sodium hydride (0.13 g, 5.4 mmol) suspended in 30 ml of anhydrous tetrahydrofuran (THF), methyl diethylphosphonoacetate (1.13 g, 5.4 mmol) was injected slowly. After stirring for 15 min, ketone **1** (5 mmol) in 15 ml of anhydrous THF was added dropwise. The reaction mixture was stirred under reflux for 10 h. After cooling to room temperature, 15 ml of water were added and the water layer was extracted with ether. The organic layer was dried over anhydrous $MgSO_4$. After removal of the solvent, the crude product was purified by silica flash column chromatography (2-hexane–ethyl acetate; 5:1. v/v). Yield: 68%. E–Z ratio=6:1. E-isomer: ^{1}H NMR, δ: 2.56 (d, 3H, ^4J=0.6 Hz, CH_3), 3.74 (s, 3H, CH_3O), 5.15 (s, 2H, CH_2O), 6.11 (q, 1H, ^4J=0.6 Hz, H-C2), 6.97 (d, 2H, J=8.8 Hz, H-C3′ and H-C5′), 7.46 (d, 2H, J=8.8 Hz, H-C2′ and H-C6′) ppm.

2.3.3. 3-[4′-(Pentafluorobenzyloxy)phenyl)crotonic acid, 1b

To a solution containing 0.5 g (1.34 mmol) of methyl 3-[4′-(pentafluorobenzyloxy)phenyl]crotonate (**1a**) in 25 ml of methanol, 25 ml of 10% sodium hydroxide were added. The mixture was stirred at room temperature for 3 h and concentrated on a rotavapor to remove most of the solvent. After adjusting the pH of the solution to 2, the mixture was extracted with ether three times. The combined organic layers were dried over anhydrous $MgSO_4$. After removal of the solvent, 1.19 g (yield: 89%) of the product were obtained.

^{1}H NMR (DMSO-d_6): E-isomer: δ: 2.50 (d, 3H, J=1.0 Hz, CH_3), 4.10 (s, 1H, OH), 5,24 (s, 2H, CH_2), 6.10 (d, 1H, J=1.0 Hz, HC=), 7.09 (d, 2H, J=8.5 Hz, HC3′ and HC5′), 7.56 (d, 2H, J=8.5 Hz, HC2′ and HC6′) ppm. Z-isomer: δ: 2.13 (d, 3H, J=1.0 Hz, CH_3), 3.68 (s, 1H, OH), 5.21 (s, 2H, CH_2), 5.87 (d, 1H, J=1.0 Hz, HC=), 7.02 (d, 2H, J=8.5 Hz, HC3′ and HC5′), 7.26 (d, 2H, J=8.5 Hz, HC2′ and HC6′) ppm. ^{13}C NMR. (DMSO-d_6): E-isomer: δ: 16.9 (CH_3), 57.4 (CH_2), 110.2 (C1″, $^2J_{F\text{-}C}$=18 Hz), 113.8 (C2), 114.7 (C3′ and C5′), 127.7 (C2′ and C6′), 134.5 (C1′), 137.1 (C2″ and C6″, $J_{F\text{-}C}$=252 Hz),141.1 (C4″, $J_{F\text{-}C}$=250 Hz), 145.2 (C3″ and C5″, $J_{F\text{-}C}$=249 Hz), 153.1 (C3), 158.5 (C4′), 167.7 (C=O) ppm. Z-isomer: d: 26.3 (CH_3), 57.3 (CH_2), 110.2 (C1″, $^2J_{F\text{-}C}$=18 Hz), 116.0 (C3′ and C5′), 117.9 (C2),128.9 (C2′ and C6′), 133.5 (C1′), 137.1 (C″2‘ and C6″, $J_{F\text{-}C}$=252 Hz), 141.1 (C4″, $J_{F\text{-}C}$=250 Hz), 145.2 (C3″ and C5″, $J_{F\text{-}C}$=249 Hz), 152.5 (C3), 157.4 (C4′), 166.9 (C=O) ppm.

2.3.4. 3-[4′-(Pentafluorobenzyloxy)phenyl]crotonate N-hydroxysuccinimide ester, ***1c***

To a mixture containing 35.8 mg (0.1 mmol) of **1b** and 13.8 mg (0.12 mmol) of N-hydroxysuccinimide in 5 ml of anhydrous dioxane at 5°C was added 24.8 mg (0.12 mmol) of N,N-dicyclohexylcarbodiimide. The reaction mixture was stirred at room temperature overnight. Removal of the formed dicyclohexylurea by paper filtration and of the solvent on a rotovapor gave 37.8 mg (83%) of product (mostly *E*-isomer; see Fig. 2) as white crystals. (Further purification was unnecessary for the next coupling step, but the product could be recrystallized from chloroform). Storage under nitrogen at −70°C stabilized the dry product for at least six months. ^{1}H NMR ($CDCl_3$), *E*-isomer, δ: 2.61 (s, 3H, CH_3), 2.87 (bs, 4H, CH_2CO), 5.15 (s, 2H, CH_2), 6.32 (s, 1H, HC=), 7.01 (d, 2H, J=8.9 Hz, HAr) 7.54 (d, 2H, J=8.9 Hz, HAr) ppm.

2.3.5. Pentafluorobenzyloxyaryl ketones ***2–16***

These compounds were prepared in the same way as **1** (Section 2.3.1), starting with commercially available phenolic ketones from Aldrich, except for ketone **8**, which was prepared as described in Ref. [10]. The structures of the compounds were confirmed spectroscopically and by the data summarized in Table 1.

2.3.6. Olefin-release group electrophore (1)-labeled DNA oligomer (E_1-DNA)

A 50-μl volume of a 5′-amino-linked DNA 20-mer (310 ng/μl), 10 μl of acetonitrile, 2 μl of 1.0 *M* sodium carbonate (pH 9.0) and 1 μl of **1c** (21 μg/μl) in acetonitrile were combined in a glass vial. After 30 min at room temperature, the product was purified by HPLC by making two injections (each containing half of the sample) and collecting the appropriate fraction, as indicated in Fig. 2. The collected, combined samples were evaporated to dryness (Speedvac), and resuspended in 1 ml of water. An absorbance measurement was made at 260 nm to quantify the probe (33 μg of single stranded DNA/1.0 AU). DNA digestion with formic acid followed by HPLC was done to confirm the identity of the product, based on its composition of nucleobases [11].

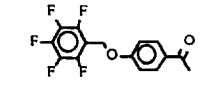
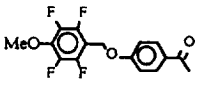
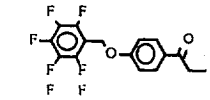
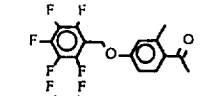
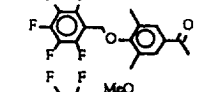
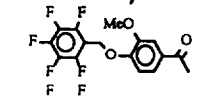
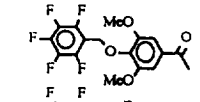
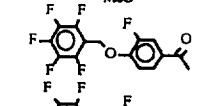
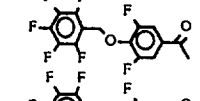
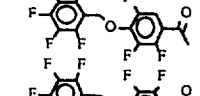
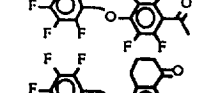
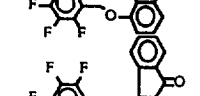
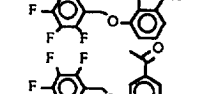
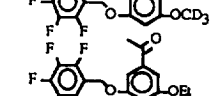
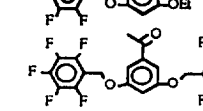
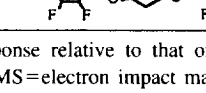
2.4. Detection of E_1-labeled DNA on nylon

To a 1-cm diameter circle of MSI nylon membrane (cleaned first by immersion in 5 ml of acetonitrile for 1 h, then air dried) was added 50 μl of 1.85 ng/μl E_1-DNA, followed after 10 min of drying by 200 μl of 0.02 *M* $KMnO_4$ and then gentle swirling for 5 min at 50°C. The released ketone was isolated by extracting the aqueous/dot sample with 500 μl of hexane. Solid phase extraction was performed as follows: a 100-mg amount of silica gel (200–300 mesh) from EM Science (Cherry Hill, NJ, USA), packed in a Pasteur pipet with Kimwipe as a frit, was washed with 1 ml of ethyl acetate and 2 ml of hexane. After the 400 μl of the hexane extract were added, followed by a wash with 1 ml of hexane, the ketone was eluted with 1 ml of ethyl acetate, evaporated (Speedvac, 25 min, no heating) and redissolved in 25 μl of ethyl acetate. A 1-μl volume was injected into a GC–EC-MS system.

3. Results and discussion

Potentially each of many different DNA oligomers can be labeled individually with a different releasable electrophore. These can then be combined to form a cocktail reagent that can be used in a multiplexed hybridization assay to detect many target DNA sequences simultaneously, based on electron capture. This requires not only that the released electrophores have different retention times by GC or different masses, but that they give comparable responses by electron capture. To demonstrate the feasibility of this approach with ketone electrophores, we synthesized compounds **1–16** (structures shown in Table 1) and tested them by both GC–ECD and GC–EC-MS. As seen in the table, all of the responses by GC–ECD are comparable (less than two-fold apart). Also, there is a good spread for their retention times by GC–ECD. As anticipated, each ketone electrophore gave a mass spectrum by GC–EC-MS which consisted of a single major ion (at M−181, from loss of a pentafluorobenzyl radical from the parent anion radical; data not shown). It is well known that O-pentafluorobenzyl phenols tend to

Table 1
Properties of ketone electrophores

No.	Compound	GC–ECD		GC–EC-MS[c]		EI–MS[d]	m.p.[e]
		Molar response[a]	t_R (min)[b]	[M–PFBz]$^-$		M^+	(°C)
1		1.0	2.77	135	316		88-89
2		1.1	7.23	135	328		104-105
3		1.0	4.14	149	330		71-72
4		1.1	3.64	149	330		120-121
5		1.1	4.61	163	344		100-101
6		1.3	4.71	165	346		110-111
7		0.9	6.01	179	360		119-120
8		0.8	2.01	153	334		99-100
9		0.9	1.15	171	352		62-63
10		0.8	0.81	189	370		76-77
11		0.8	0.69	207	388		76-77
12		1.2	6.82	161	342		79-80
13		0.9	10.55	195	376		126-127
14		1.5	5.34	168	349		67-68
15		1.4	6.38	179	360		66-67
16		1.2	10.99	331	331[f]		102-103

[a] Response relative to that of **1**. [b] See Section 2 for GC conditions. [c] PFBz=pentafluorobenzyl (which has a relative mass of 181 u). [d] EI–MS=electron impact mass spectrometry. [e] Uncorrected. [f] *m/z* 331 is M–PFBz for **16**.

undergo electron capture in this way [12]. Table 1 also provides the molecular masses of the compounds, as confirmed experimentally by electron impact MS (aside from compound **16**, where M−181 but not M is observed) and their melting points. Thus, it is clear that a multiplicity of ketones can be prepared with good detection properties by both GC–ECD and GC–EC-MS.

We next set out to demonstrate some feasibility for a ketone electrophore as a releasable label on DNA. The overall scheme for the experiment that we accomplished is presented in Fig. 1. We began by synthesizing compound **1c**, an olefin-release group electrophore, *N*-hydroxysuccinimide ester. This compound was obtained as a mixture of olefin isomers (*E*/*Z*=6:1, established both by NMR for the precursor acid and by HPLC analysis of **1c**, as shown in Fig. 2). Reaction of **1c** with a 5′-aminoalkyl DNA 20-mer under aqueous conditions gave an olefin release group electrophore-labeled DNA oligomer (E_1-DNA). The reaction could be conveniently monitored by reversed-phase HPLC, as shown in Fig. 3. As anticipated, the electrophore oligomer, due to the presence of the non-polar electrophore, elutes significantly later than the starting oligomer. Also seen in the chromatogram are the peaks for unreacted DNA, unreacted 1c, isomeric olefin acids **1b** (hydrolysis products of **1c**) and N-hydroxysuccinimide. Trityl oligomers derived from DNA synthesis similarly elute later by reversed-phase HPLC relative to the corresponding unlabeled oligomer [13,14].

Hybridization reactions with DNA probes are frequently performed on a nylon membrane where the target DNA (to be detected) typically has been blotted directly, or after prior electrophoretic separation on a polyacrylamide gel. Thus, we tested the E_1-DNA by applying 1.3 pmol onto a dot of nylon membrane. After the sample had dried, the dot was oxidized with aqueous potassium permanganate to release the electrophore as ketone **1**. Extraction of the ketone from the aqueous phase, with hexane, and purification by silica solid-phase extraction was done prior to injection of an aliquot into a GC–EC-MS system. The resulting mass chromatogram, based on selected-ion monitoring of the acetophenolate anion arising from dissociative electron capture of **1**, is shown in Fig. 4. External calibration with authentic **1**

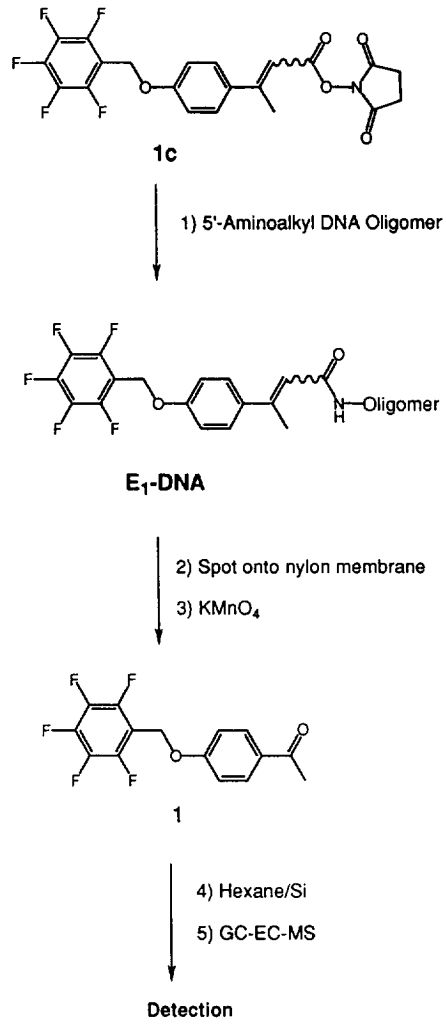


Fig. 1. Scheme for the preparation and detection of an olefin-release group electrophore-labeled DNA oligomer (E_1-DNA) spotted on nylon.

showed that the yield of ketone from the electrophore-labeled oligomer dotted onto the nylon membrane was essentially quantitative.

This methodology thereby establishes conditions for the preparation and detection of electrophore-labeled oligomers as DNA probes, relevant to hybridization assays on nylon. High sensitivity is anticipated ultimately, for three reasons. First, the release and recovery of the ketone electrophore is

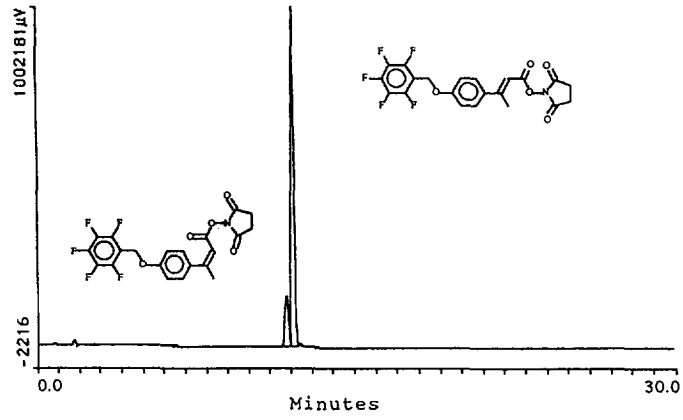


Fig. 2. HPLC chromatogram for isomers (*Z*)**1c** and (*E*)**1c** obtained after injecting 10 μl of 2.9 μg/μl (total solute) in acetonitrile and monitoring the absorbance at 273 nm. Column: LCPCN 5-8378 (cyanopropyl–dimethylsilyl silica, 5 μm, 250×0.46 mm; Supelco, Bellefonte, PA, USA). Mobile phase: 20 min gradient at 2 ml/min from 100% water to 100% acetonitrile. AUFS=1.0.

highly efficient. Second, the ketone electrophore gives a high response by GC–EC-MS. Finally, the nylon membrane containing the electrophore DNA probe can be washed as needed with a non-polar organic solvent to remove any background interferences, prior to release of the ketone electrophore.

In our future work, we will be extending this methodology by preparing additional electrophore-labeled DNA probes and testing them as cocktails (mixture of electrophore-labeled probes) in hybridization assays to more fully explore their potential for high multiplicity and sensitivity.

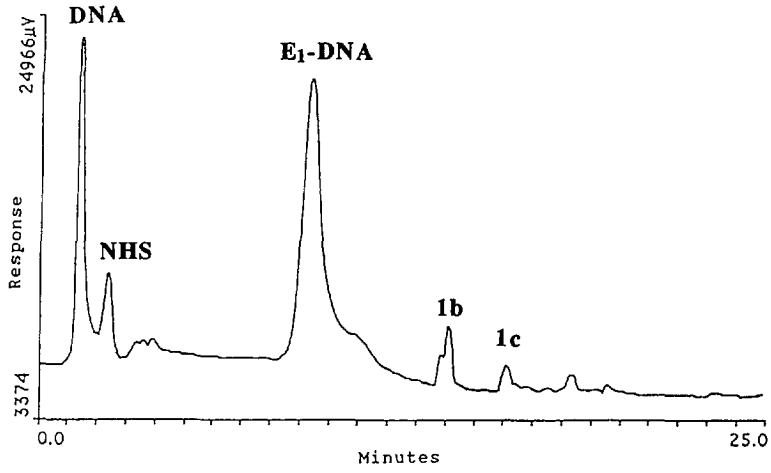


Fig. 3. Reversed-phase HPLC chromatogram for the reaction mixture described in Section 2 that gave E_1-DNA (step 1 in Fig. 1). One-half (25 μl) of the mixture was injected. Mobile phase: acetonitrile–H_2O (1:99, v/v). AUFS=0.025. Column: Brownlee Spheri-5, RP-18 (220×4.6 mm I.D., 5 μm) HPLC column, equipped with a Brownlee RP-18 (43.2×15 mm I.D., 7 μm) guard column, bought from Applied Biosystems (San Jose, CA, USA).

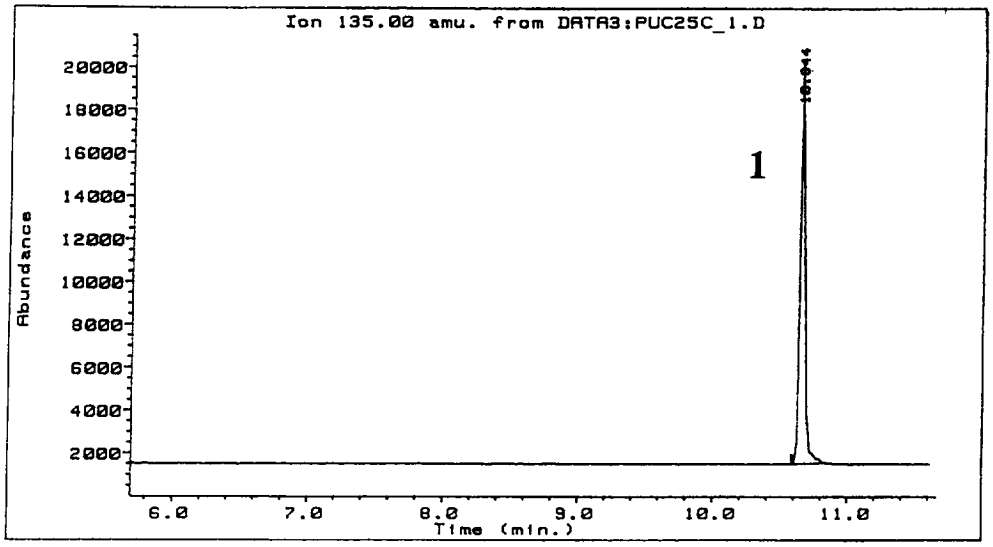


Fig. 4. GC–ECD-MS chromatogram of ketone **1** released from an olefin-release group electrophore DNA oligomer (E_1-DNA) spotted onto a nylon membrane (steps 2–5 in Fig. 1).

Acknowledgments

This work was supported by NIH Grant HG00029. Contribution No. 687 from the Barnett Institute.

References

[1] S. Agrawal (Editor), Protocols for Oligonucleotide Conjugates, Humana Press, Totawa, NJ, 1994.
[2] E. Schröck, S. du Manoir, T. Veldman, B. Schoell, J. Wienberg, M.A. Ferguson-Smith, Y. Ning, D.H. Ledbetter, I. Bar-Am, D. Soenksen, Y. Garini and T. Ried, Science, 273 (1996) 494.
[3] P. Richterich and G.M. Church, Methods Enzymol., 218 (1993) 187.
[4] R.W. Giese, Trends Anal. Chem., 2 (1983) 166.
[5] R. Joppich-Kuhn, M. Joppich and R.W. Giese, Clin. Chem., 28 (1982) 1844.
[6] S. Abdel-Baky, K. Allam and R.W. Giese, Anal. Chem., 65 (1993) 498.
[7] S. Abdel-Baky, N. Klempier and R.W. Giese, Tetrahedron, 17 (1990) 5859.
[8] W. Clark Still, Acc. Chem. Res., 29 (1996) 155.
[9] P.F. Britt, G.B. Hurst and M.V. Buchanan, J. Mass. Spectrom., 31 (1996) 661.
[10] L. Xu and R.W. Giese, J. Fluor. Chem., 67 (1994) 47.
[11] D.H. Fisher and R.W. Giese, J. Chromatogr., 452 (1988) 51.
[12] N. Chalaux, J.M. Bayona and J. Albaigés, J. Chromatogr. A, 686 (1994) 275.
[13] T.L.A. Hill and J.W. Mayhew, J. Chromatogr., 512 (1990) 415.
[14] T. Brown and D.J.S. Brown, Methods Enzymol., 211 (1992) 20.