Alkyne Metathesis Graft Polymerization: Synthesis of Poly(ferricinium)-Based Silica Supports for Anion-Exchange Chromatography of Oligonucleotides

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ABSTRACT: 2-[3-(Ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (**2**) was prepared via Wittig- and chloro-Wittig-type reactions. **2** reacts with the well-defined Schrock-initiator Mo(*N*-2,6-$Me_2C_6H_3$)($CHCMe_2Ph$)($OCMe(CF_3)_2)_2$ via α-addition. The resulting class VI polymerization system allows the living polymerization of **2** up to a degree of polymerization of 150. Mesoporous and nonporous silica (Nucleosil 300-5 and Micra, respectively) with different specific surface area (100 and 2 m^2/g, respectively) were surface-derivatized with norborn-2-en-5-yltrichlorosilane, leading to an average surface functionalization of 230 and 50 μmol norbornene/g, respectively. Monomer **2**, ethynylferrocene (**1**), and 2-[4-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene) (**3**) were surface-grafted onto these silica supports via alkyne metathesis polymerization using Mo(*N*-2,6-$Me_2C_6H_3$)($CHCMe_2Ph$)($OCMe(CF_3)_2)_2$. Typical amounts of grafted monomer were in the range 50−5 μmol/g. The resulting poly(ferrocene)-grafted supports were subsequently oxidized with iodine to the corresponding poly(ferricinium)-grafted supports and successfully used for anion-exchange chromatography of oligonucleotides $(dT)_{12}$−$(dT)_{18}$.

Introduction

The synthesis of oligonucleotides represents an important field in molecular biology. They are widely used in hybridization experiments, as primers in the PCR reaction as well as adapters for the construction of deletions, insertions, and other biologically relevant mutations.[1] Since oligonucleotides synthesized by standard solid-phase synthesis are usually contaminated with failure sequences and partially deprotected sequences, purification and careful quality control are obviously mandatory. Because of the ease of automatization, detection, and quantification, oligonucleotides are usually separated by high-performance liquid chromatography (HPLC) techniques such as ion-pair reversed-phase,[1,2] mixed-mode,[3] size-exclusion,[4] and anion-exchange HPLC.[5,6] Generally speaking, LC analyses of biopolymers are characterized by a comparably slow mass transfer resulting from small diffusion coefficients and by multimodal interactions between the analyte and the stationary phase. Consequently, macroporous particles are used in order to facilitate diffusion by providing large pores. Alternatively, totally nonporous particles lacking any accessible intraparticle pores have been proposed to enhance mass-transfer kinetics. Unfortunately, such supports are characterized by comparably low numbers for the specific surface area (σ), resulting in unfavorable low loading capacities of analytes.

So far, we independently performed mechanistic studies on the controlled synthesis of metallocene-containing poly(acetylenes)[7−11] and application-oriented syntheses of surface-derivatized supports.[12−14] In the course of our investigations on anion-exchange supports, we were interested as to which extent these two areas might be combined in a useful way. Ferrocenes and poly(ferrocenes) represent stable compounds yet may easily and in certain cases reversibly be oxidized to the corresponding ferricinium and poly(ferricinium) derivatives.[10,15] Due to this peculiar feature, these compounds have already been of certain interest for battery fabrication,[16] redox-active surfaces,[17] and electrodes.[18] Nonetheless, there are no reports so far on the use of ferricinium-based ligands as functional groups for anion exchange since in anion-exchange chromatography[6,19−21] amino-based stationary phases are used almost exclusively. In due consequence, we investigated as to which extent "tentacle-type" poly(ferricinium)-grafted mesoporous silica prepared by a "grafting-to approach" may be used in anion-exchange chromatography of biopolymers. In this contribution, we report on the successful combination of poly(alkyne) and surface-grafting chemistry both based on metathesis techniques.

Results and Discussion

Synthesis of Monomers. The structures of compounds **1**−**3** are summarized in Figure 1.

Optimized syntheses for **1** from ferrocene aldehyde triphenyl(chloromethyl)phosphonium bromide[22] and for **3**[11] have been described previously. The synthesis of the terminal, octamethylferrocene-substituted acetylene, 2-[3-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene) (**2**), may be accomplished by three different well-established Wittig[23,24] sequences (Scheme 1), yet only one allows its preparation in acceptable yields. The first approach entails the reaction of octamethylferrocene carbaldehyde with 3-iodobenzyltriphenylphosphonium bromide under basic conditions. Unfortunately, the resulting compound **5** is only formed in 12% yield and represents a 1:1 mixture of the *E*- and *Z*-isomer. Alternatively, octamethylferrocenylmethyl-

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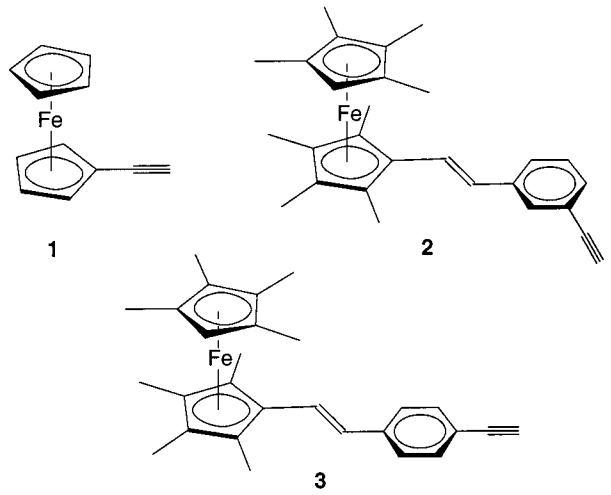


Figure 1. Structures of compounds **1**−**3**.

triphenylphosphonium bromide may be reacted with an excess of isophthaladehyde to yield **6**, which may subsequently be converted to **3** via well-established chloro-Wittig[22,25] sequences. Since these transformations are comparably expensive and involve a significant number of synthetic steps, the optimum approach for **2** represents the reaction of octamethylferrocenylmethyltriphenylphosphonium bromide with 3-bromobenzaldehyde to yield **7** in 67% yield. In this context it is noteworthy that exclusively the *E*-isomer is formed. This compound is finally converted into **2** via consecutive Sonogashira−Hagihara coupling and base-promoted deprotection reactions in 56% overall yield.

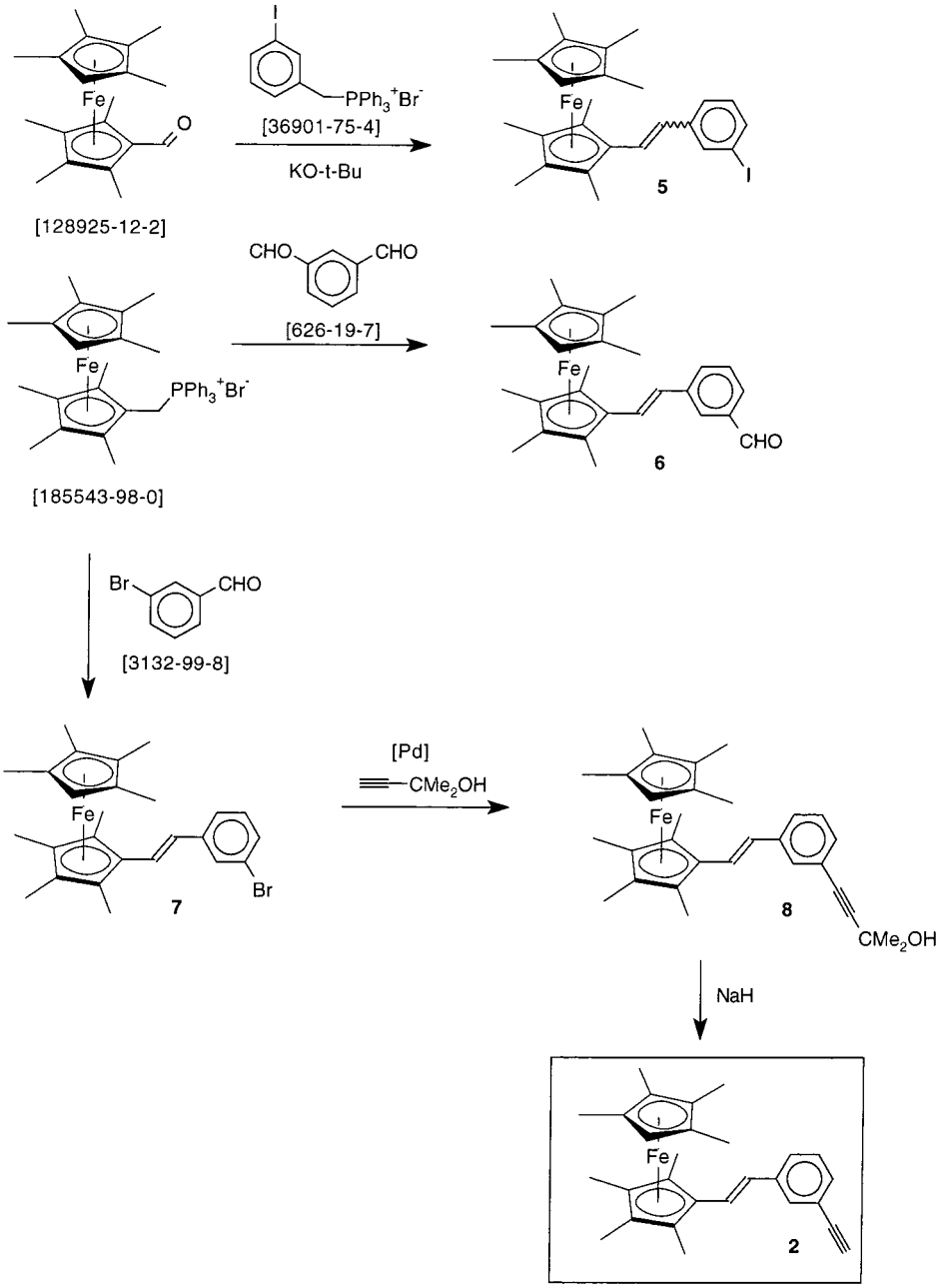
Compounds **1**−**3** (Figure 1) are polymerized in a controlled living manner[26] by the metathesis initiator Mo(*N*-2,6-$Me_2C_6H_3$)(CHCMe_2Ph)(OCMe(CF_3)$_2$)$_2$. Linear plots (R^2 > 0.95) of *N* (number of equivalents) and molecular weight are obtained throughout. It is worth mentioning that polymerizations proceed by two different mechanisms. Thus, the polymerization of compounds **1**[7] and **2** proceeds via α-insertion, while compound **3** undergoes selective *β*-insertion as indicated by a new alkylidene signal for H_α at $\delta = 12.7$ ppm and the lack of any coupled signals for H_β and H_γ.[11] Evidence for the fact that compound **2** adds to the initiator via repetitive α-insertion is provided by the complete lack of any signal for a propagating α-alkylidene proton and by the appearance of two sets of signals for coupled trans-configured double bonds (see Experimental Section) that form in the course of the α-insertion and may be attributed to the first and second insertion product. The reason for the different insertion modes with one initiator obviously lies in the different steric requirements of the three ligands. Thus, the "small" molecule ethynylferrocene as well as the meta-substituted octamethyl derivative allows α-insertion, while the rigid para-substituted derivative requires *β*-insertion in order to avoid steric interaction with the alkoxides and the methyl groups of the imido ligand (Scheme 2).

The different mechanism obviously also accounts for the different classes of livingness[27] these compounds may be attributed to. Compounds **1**[7] and **3**[11] fulfill the requirements of a class VI and class IV living system, respectively. To estimate the quality of livingness of the polymerization of **2**, a block copolymer of **2** with norbornene was prepared 60 h after initiation of **2** with the Schrock carbene (see Experimental Section). GPC investigations on this copolymer revealed one single signal (M_n = 128 300, PDI = 1.54). On the basis of the definitions of this classification, this suggests that at least 90% of the initiator were active at the time where norbornene was added, thus suggesting a class VI living system for **2**. The enhanced stability of the polymerizations proceeding via α-insertion may be expected and is best explained by the higher stability of α,α-disubstituted molybdenum alkylidenes. In due consequence, higher degrees of polymerization (DP) are obtained in polymerizations based on α-addition. The DP of compound **1** is limited to roughly 70 for solubility reasons; nevertheless, compound **2** may be prepared up to a DP of at least 150 (M_w = 64 700, PDI = 1.21). In contrast, the polymerization of monomer **3** is limited to a DP of roughly 60 with polydispersities ≤1.5.

Synthesis of Supports. In principle, three different approaches may be applied to the synthesis of ferricinium-derivatized silica. The first entails the covalent binding of a suitable spaced ferrocene derivative using standard silica chemistry. Despite the ease of such an approach, it requires the use of macroporous, low surface area supports with all their disadvantages for the present purpose (vide supra). Complementarily, suitably functionalized, soluble prepolymers may be considered as useful for coating purposes,[28] yet again low specific surface areas and pore volumes significantly lower the attractiveness of this approach. Finally, surface-grafted polymer supports may be prepared. Such grafted supports are best prepared from endcapped silica that additionally contains sufficient polymerizable "anchoring groups" that allow the attachment of polymers via grafting techniques. If carried out in an appropriate manner, the original surface area and pore volume are preserved, and a sufficient amount of functional groups is provided. Generally, such grafting procedures may be accomplished using standard radical polymerization. Nevertheless, in contrast to free-radical polymerization,[29] metathesis-based techniques such as ADMET or alkyne polymerization[30] offer an enhanced control over molecular architecture during polymer synthesis and allow the presence of a large variety of functional groups. Thus, the length of the graft chains is controlled by the degree of polymerization (DP), and the resulting polymers are additionally uniform in terms of polydispersity (PDI < 1.5). Since monomers **1**−**3** may be polymerized in a living manner, the DP of the graft polymer may simply be varied by the stoichiometry of the corresponding monomer and the initiator (Mo(*N*-2,6-$Me_2C_6H_3$)(CHCMe_2Ph)(OCMe(CF_3)$_2$)).

Surface Grafting of Silica. In a first step, the corresponding silica was surface-modified with norbornen-2-en-5-yltrichlorosilane. This procedure provides the necessary anchoring groups. The first silica used for these purposes, Nucleosil 300-5, possesses a specific surface (σ) of 100 m^2/g. Consequently, a large amount of norborn-2-ene groups of up to 0.23 mmol/g may be introduced. As expected, the analogous reaction with nonporous Micra material (σ = 2 m^2/g) results in comparably low values of 47.6 μmol norbornene/g. Nevertheless, both materials are suitable for grafting. Thus, the attachment of monomers **1**−**3** was accomplished by combining alkyne and ring-opening metathesis graft polymerization. Polymerizations were quantitatively initiated using Mo(*N*-2,6-$Me_2C_6H_3$)(CHCMe_2-Ph)(OCMe(CF_3)$_2$) as the initiator. The resulting linear, "living" polymer chains were subsequently grafted onto the support via copolymerization with the surface-

Scheme 1. Synthesis of Compound 2

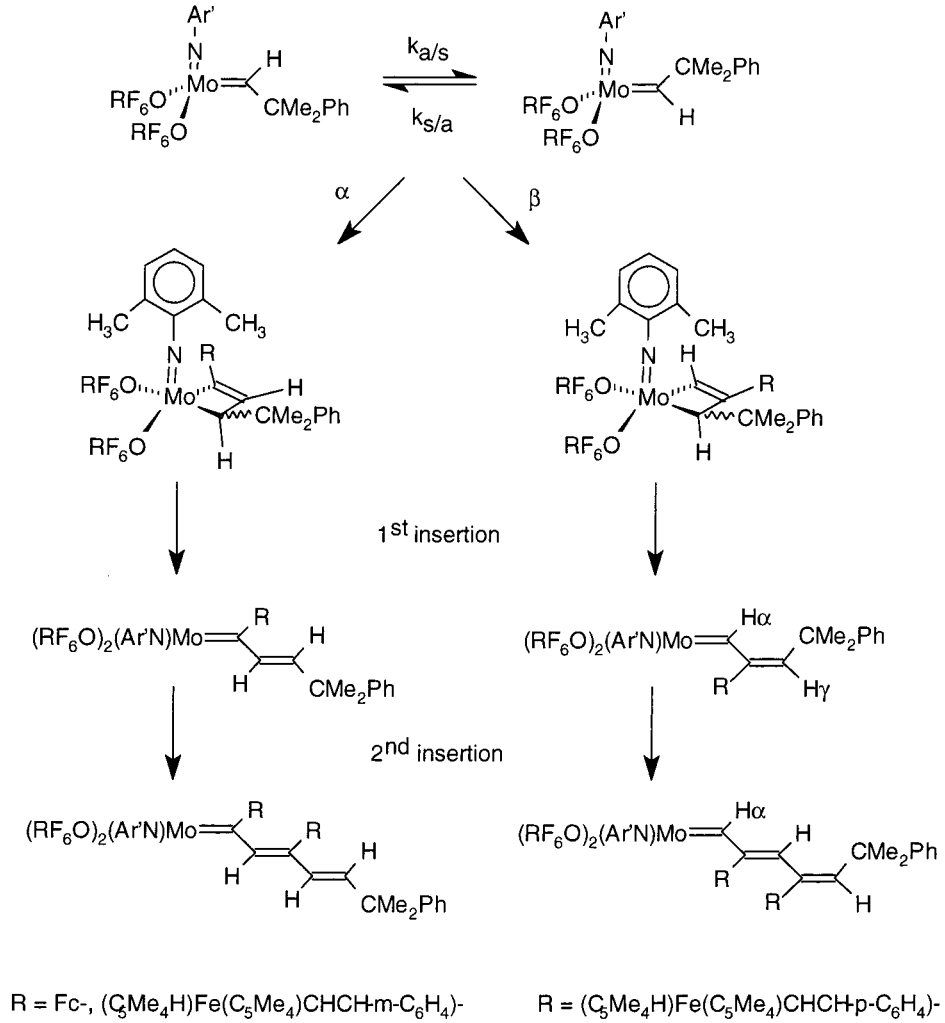


attached norborn-2-ene units. The materials that are formed by this process possess a typical tentacle-type structure. This tentacle-type structure was chosen for two reasons. On one hand, such structures generally facilitate mass transfer within the interphase.[31] On the other hand, such an approach is necessary in order to provide a sufficient amount of functional groups in particular on nonporous carriers. Keeping solubility restrictions and the classes of livingness in mind, the number of repetitive units within the tentacles was 20 for monomers **1** and **3** (supports **I** and **III**) and 40 for monomer **2** (support **II**).

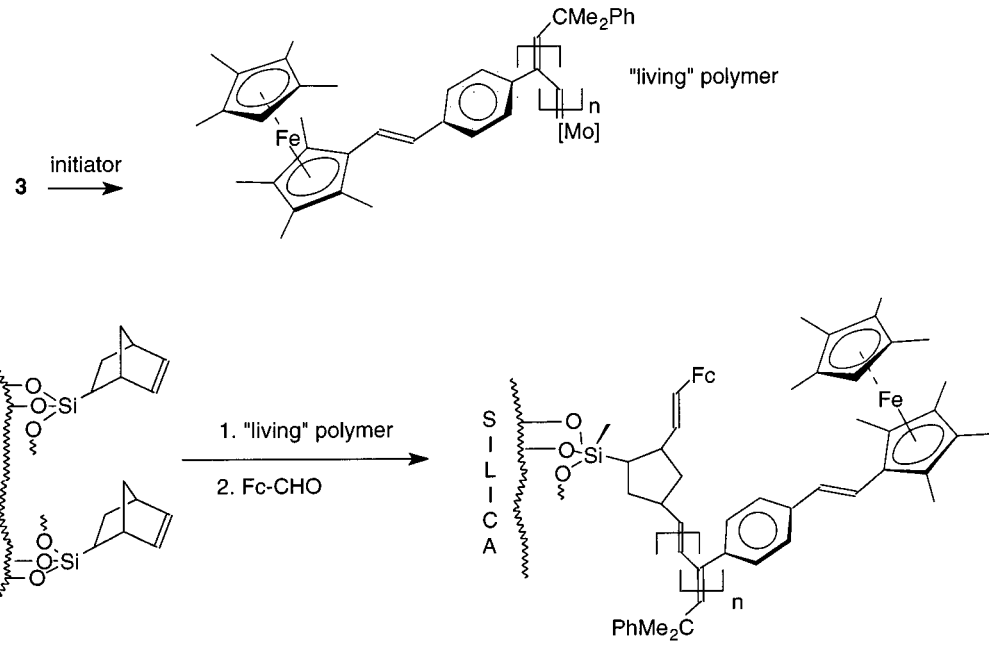
Oxidation of Grafted Supports. Ferrocenes represent 18-electron compounds that may be (reversibly) oxidized to the corresponding ferricinium derivatives. The redox potential of ferrocenes (parent redox couple Fc/Fc^+ vs silver reference = −0,45 V) strongly depends on the nature and number of substituents.[15] Thus, decamethylferrocene possesses a redox potential of +0.10 V. Because of their stability, the related octamethylferricinium-based ligands appear attractive as novel functional groups in anion-exchange chromatography. Treatment of supports **I**−**III** with a solution of iodine in acetonitrile yields the corresponding poly(ferricinium)-based stationary phases. The actual amount of polymer grafted onto the surface may easily be determined by either Fe analysis (AAS) or preferably iodine analysis, since every ferricinium unit is expected to possess a triiodide counteranion. To render the stationary phase ready for anion-exchange chromatography, the triiodide counterion may easily be replaced by any suitable anion, e.g., chlorides, sulfates, phosphates, or hexafluorophosphates.[32] A schematic drawing of the entire grafting and oxidation process is shown in Scheme 3.

Anion-Exchange Chromatography of Oligonucleotides. Poly-**1**-grafted Nucleosil 300-5 may successfully be used for the separation of synthetic oligonucleotides as demonstrated in Figure 2a by the anion-exchange separation of homologous oligodeoxythymidylic

Scheme 2. Different Modes of Insertion for Compounds 1−3



Scheme 3. Synthesis of Poly-3-Based Supports; Fc = Ferrocene, [Mo] = Mo(*N*-2,6-Me$_2$-C$_6$H$_3$)(OCMe(CF$_3$)$_2$)$_2$



acids. Gradients of sodium chloride can be applied to elute the oligonucleotides from the anion-exchange stationary phase. Additionally, solvophobic interactions between the nucleobases and the hydrophobic polymer backbone are efficiently suppressed upon the addition of 20% acetonitrile to the eluent. Under these chromatographic conditions, retention and separation of oligonucleotides according to their number of nucleotide

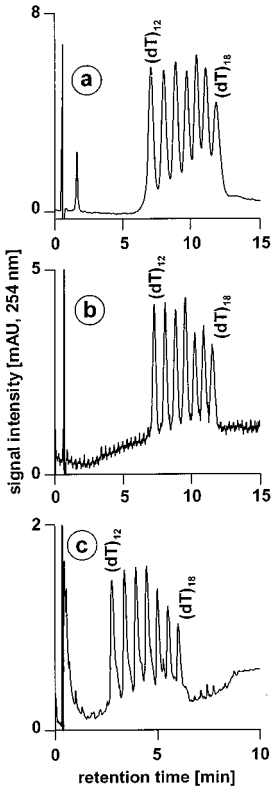


Figure 2. Chromatographic separation of a $(dT)_{12}-(dT)_{18}$ homooligonucleotide ladder on polyferricinium-based anion exchangers. Conditions in (a): column I (60 × 2.0 mm i.d. ferricinium-derivatized Nucleosil 300-5), gradient, 0.050−0.25 M sodium chloride in 20 mM Tris-HCl, pH 8.0, 20% acetonitrile in 15 min, flow rate 250 μL/min; temperature, ambient; detection, UV (254 nm); sample, 0.25 μg $(dT)_{12}-(dT)_{18}$. Conditions in (b): column II (60 × 2.0 mm i.d. octamethylferricinium-derivatized Nucleosil 300-5), gradient, 0−0.175 M ammonium sulfate in 20 mM ammonium phosphate, pH 7.0, 20% acetonitrile ACN in 15 min, flow rate 250 μL/min; other conditions as in (a). Conditions in (c): column III (60 × 2.0 mm i.d. octamethylferricinium-derivatized Micra); other conditions as in (b).

units as shown in Figure 2a represents a clear proof for a separation due to electrostatic interactions characteristic for an anion-exchange mechanism. Nevertheless, because of relatively large peak widths at half-height in the range of 0.43−0.50 min, the complete separation of all seven components of the oligonucleotide standard is not feasible. As expected, the chemical stability of supports based on polymeric unsubstituted ferricinium systems is rather low as evidenced by a significant loss of resolving power of the stationary phase of approximately 50% within 2−3 days. This fast deterioration stems from some particular features of ferrocene systems. The parent ferricinium cation is sensitive toward oxygen in solution, leading to fast decomposition, particularly in polar solvents, forming inorganic iron compounds.[33] This finally results in polyene degradation as evidenced by significantly lowered Fe contents of such supports.

The problem of stationary phase degradation may be alleviated by replacing the ferrocene moiety by an octamethylferrocene group, which is easily oxidized to the corresponding octamethylferricinium systems. Such highly methylated species are stable toward oxygen both under acidic and basic conditions.[10] In fact, the use of the more stable octamethylferricinium derivatives allows the synthesis of comparably stable separation media with excellent separation properties for synthetic oligonucleotides. Figure 2b illustrates the analysis of the oligonucleotide standard on a mesoporous Nucleosil support derivatized with octamethylferricinium utilizing an eluent that comprises 20 mM phosphate buffer, pH 7.20, and 20% acetonitrile and gradient elution with 0−0.18 M ammonium sulfate. With the octamethylferriciumium-based stationary phase, the use of ammonium sulfate enabled a significant reduction in the peak width at half-height to 0.24−0.28 min, which facilitated the complete resolution of $(dT)_{12}-(dT)_{18}$. This improvement in separation efficiency with ammonium sulfate as gradient former is not observable with the unsubstituted ferricinium-grafted stationary phase, most probably because of deterioration of the stationary phase during the course of comparative experiments in the latter case. The separation depicted in Figure 2b compares favorably with respect to elution times and peak resolution to the separation of an oligothymidylic acid ladder on a commercially available silica-based anion exchanger (Partisil PXS 10/25 SAX from Whatman).[34]

In an attempt to increase speed of analysis for oligonucleotides, we grafted the octamethylferricinium stationary phase onto a nonporous silica support. The separation efficiency that may be achieved with nonporous Micra-based materials clearly stems from the tentacle-grafting approach, which provides sufficient derivatization in the absence of any pores, resulting in enhanced mass-transfer kinetics and improved column performance. Thus, as evidenced by elemental analysis, surface-derivatized Micra possesses a functional group density (expressed in mmol/m^2) that is 10 times higher than that of grafted Nucleosil (Table 1). The improvement in separation speed is verified by the chromatogram shown in Figure 2c, where the fractionation of $(dT)_{12-18}$ is accomplished within less than 7 min. This represents a reduction in analysis time by almost a factor of 2 with slightly improved peak resolution (peak widths at half-height between 0.15 and 0.20 min) compared to the separation on the mesoporous stationary phase (Figure 2b). Moreover, the separation performance achieved with the nonporous octamethylferricinium material comes very close to that described for the separation of $(dT)_{12-18}$ on polyethylenimine-coated nonporous silica (PEI-silica, Figure 2 in ref 1) or to the separation of $p(dT)_{12-18}$ on a commercial, nonporous diethylaminoethyl polymer-based stationary phase (DEAE-NPR from TosoHaas, Figure 1 in ref 1).

However, conjugatively connected octamethylferricinium termini may undergo consecutive side reactions involving the formation of α-cations generated by protonation of the olefinic linker (Scheme 4). In the case of octamethylferriciumnium-derivatized stationary phases, columns can be used for 7−10 days, before a slow yet observable deterioration of the stationary phase results in loss of column performance. Current research therefore focuses on nonconjugatively connecting of the cationic functionalities.

Experimental Section

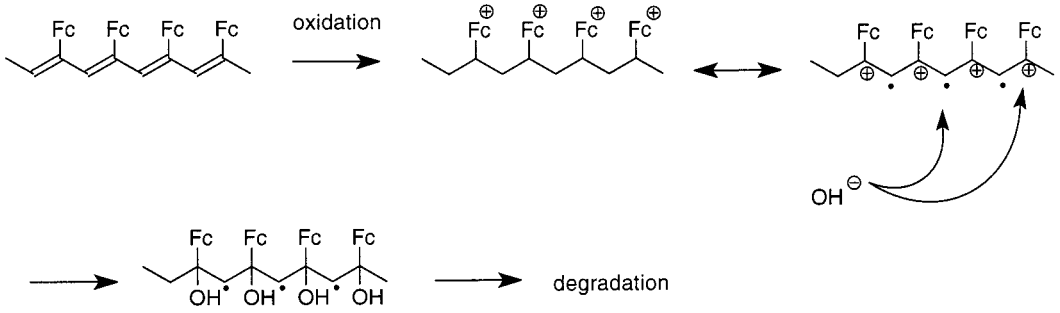
All experiments were performed in a nitrogen-mediated drybox (Braun, Garching, Germany) or by standard Schlenk techniques. General statements on standard procedures or on equipment and methods have been made previously.[35,36] ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer; mass spectra were measured on a Varian C7 (EI, 70 eV) mass spectrometer and on a Finnigan

Table 1. Properties of Metallocene-Derivatized Supports

	silica	NBE groups [μmol/g]	monomer	tentacle length (N)[c]	μmol monomer/g (μmol monomer/m^2)
I	Nucleosil 300-5[a]	230	1	20	50 (0.5)
II	Nucleosil 300-5[a]	230	2	40	30 (0.3)
III	Micra[b]	47.6	3	20	4.5 (2.25)

[a] 100 m^2/g. [b] 2 m^2/g. [c] N is the number of monomers.

Scheme 4. α-Carbocation Formation and Polymer Degradation



MAT, 95 S (FAB, ESI, and high-resolution mass spectra), respectively. Melting points were obtained on a Leica VM TG hot-plate apparatus and are corrected. Atomic absorption spectroscopy experiments (AAS) were carried out on a Phillips PU-7000. Purchased starting materials, HPLC solvents, and the investigated compounds were used without any further purification. HPLC-grade water was prepared by E-Pure (Barnstead) and used throughout. Ethynylferrocene [1271-47-2],[22] 2-[4-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene),[11] and Mo(N-2,6-$Me_2C_6H_3$) ($CHCMe_2Ph$)($OCMe(CF_3)_2$)$_2$ [154002-56-9][37] were synthesized as described in the literature and checked for purity by means of NMR. Silica materials (Micra, 1.5 μm, nonporous, specific surface = 2 m^2, Nucleosil 300−5, 5 μm, 300 Å, specific surface = 100 m^2) were purchased from Bischoff (Leonberg, Germany) and Macherey-Nagel (Düren, Germany). Norborn-2-en-5-yltrichlorosilane (ABCR, Darmstadt, Germany) was used for silanization throughout. The final norborn-2-ene content was determined via elemental analysis and was 47.6 μmol/g (Micra) and 1 0.23 mmol/g (Nucleosil 300-5). Stationary phases were slurry-packed into 60 × 2 mm i.d. columns (GROM, Germany). The oligonucleotide standard $(dT)_{12}$−$(dT)_{18}$ was purchased from Pharmacia. A Knaur high-pressure HPLC packing pump was used for column packing. HPLC experiments were carried out on a high-performance liquid chromatograph consisting of two gradient pumps (model M480, Gynkotek, Germering, Germany), a vacuum degasser (Knauer, Berlin, Germany), and a UV detector (UVis 205 or UVis 200, Linear Instruments, Fremont, CA). Data were collected and processed using a chromatography data system (Dionex-Softron, Germering, Germany).

***(E)*-[2-(3-Bromophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (7).** A Schlenk vessel was charged with (1′,2,2′,3,3′,4,4′,5-octamethylferrocenyl)methyltriphenylphosphonium bromide ([185543-98-0], 6.820 g, 10.0 mmol) and 150 mL of anhydrous THF. The suspension was cooled to −50 °C (ethanol/liquid nitrogen cooling bath). Afterward, potassium *tert*-butylate ([865-47-4], 1.350 g, 12.0 mmol, 1.2 mequiv) was added all at once. The cooling bath was replaced by an ice/water cooling bath, and the deep red mixture was stirred for 30 min at 0 °C. Subsequently, 3-bromobenzaldehyde (2.030 g, 11.0 mmol, 1.1 mequiv) [3132-99-8] was added, and the mixture turned light red. The cooling bath was removed, and after stirring for 1 h at room temperature, the mixture was quenched with 30 mL of water, the THF was removed, and the resulting suspension was extracted with 100 mL of ether. The ether layer was washed with 50 mL of water and 30 mL of brine. After drying with Na_2SO_4, the ether was evaporated. The crude product was purified by column chromatography (basic alumina, 5% H_2O, Aldrich 5016 A, Brockmann III, 3 × 30 cm, *n*-hexane). Yield: 3.191 g (67%) of a red solid, stable on air; mp 95−97 °C (corrected with benzil). ^{1}H NMR ($CDCl_3$): δ 1.62 (6H, s, 2 × CH_3 of cp), 1.68 (6H, s, 2 × CH_3 of cp), 1.77 (6H, s, 2 × CH_3 of cp), 1.94 (6H, s, 2 × CH_3 of cp), 3.25 (1H, s, of cp), 6.58 (1H, d, ^3J = 16.58, E-HC=CH), 6.86 (1H, d, ^3J = 16.58, E-HC=CH), 7.09, 7.25, 7.32, 7.56 (4H, m, phenyl). ^{13}C NMR ($CDCl_3$): δ 9.19, 9.72, 11.02, 11.09 (*C*H$_3$), 71.18, 79.18, 80.38, 80.57, 81.79 (ferrocene), 122.83, 123.56, 124.52, 127.94, 128.78, 128.91, 129.98 (phenyl, C=C). IR (KBr): 2902 (ν_{st-CH}), 1627 ($\nu_{st-C=C}$), 1590 (Ar_{C-C}), 1555(Ar_{C-C}), 1371 ($\nu_{\delta-CH3}$), 1028 (ferrocene), 959 (ν_{st-CH3}) cm^{-1}. MS (FAB) for $C_{26}H_{31}BrFe$ (M = 479.28 g/mol): m/z 478.0 (100%).

***(E)*-[2-(3-(2-Hydroxy-2-methyl-butyn-1-yl)phenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (8).** A Schlenk vessel was charged with (*E*)-[2-(3-bromophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (1.000 g, 2.09 mmol) and with 150 mL of dry 2-propylamine. The solution was ultrasonicated for 10 min. Afterward, 2-methyl-3-butin-2-ol ([155-19-5], 1.0 mL, 5 mequiv) was added. Subsequently, small portions of $(Ph_3P)_2PdCl_2$ (30 mg) and CuI (20 mg) were added, and the mixture was refluxed for 12 h. The solvent was removed, and the resulting suspension was extracted with 100 mL of ether. The ether layer was washed once with 50 mL of an ammonium chloride solution and once with water. After drying with Na_2SO_4, the ether was evaporated. The crude product was purified by column chromatography (Silica gel, 3 × 15 cm, CH_2Cl_2/*n*-hexane 1/1). The product was collected as a red liquid. Yield: 0.527 g (59%). ^{1}H NMR ($CDCl_3$): δ 1.64 (6H, s, 2 × CH_3 of cp), 1.71 (6H, s, 2 × CH_3 of cp), 1.80 (6H, s, 2 × CH_3 of cp), 1.96 (6H, s, 2 × CH_3 of cp), 2.41 (1 H; s, −OH), 3.29 (1H, s, CH of cp), 6.62 (1H, d, ^3J = 16.58, E-HC=CH), 6.87 (1H, d, ^3J = 16.58, E-HC=CH), 7.20, 7.25, 7.37, 7.40, 7.48 (4H, m, phenyl protons). ^{13}C NMR ($CDCl_3$): δ 9.26, 9.79, 11.09, 11.13 (*C*H$_3$), 31.44 (CH_3), 65.51 (C_{ipso} of C−OH), 71.25, 77.50, 79.28, 80.55, 80.72, 81.83, 82.18, 93.59 (ferrocene, CCH), 122.97, 125.03, 125.53, 128.46, 128.51, 129.34, 138.94 (phenyl, C=C). IR (KBr): 2968 (ν_{st-CH3}), 2901 (ν_{st-CH}), 1620 ($\nu_{st-C=C}$), 1593 (Ar_{C-C}), 1574 (Ar_{C-C}), 1376 ($\nu_{\delta-CH3}$), 1166 (ferrocene), 1028(ferrocene), 958 (ν_{st-CH3}).

***(E)*-[2-[3-(Ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (2).** (*E*)-[2-(3-(2-hydroxy-2-methyl-butyn-1-yl)phenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (0.500 g, 1.04 mmol) was dissolved in 30 mL of dry toluene, and 500 mg of sodium hydride was added. The solution was refluxed for 120 min and then cooled to room temperature Afterward, the solvent was removed, and the resulting suspension was extracted with 100 mL of diethyl ether. The ether layer was washed with 50 mL of water. After drying over Na_2SO_4, the ether was evaporated, and the product was dried in vacuo (red liquid). Yield: 0.420 g (95%). ^{1}H NMR ($CDCl_3$): δ 1.59 (6H, s, 2 × CH_3 of cp), 1.64 (6H, s, 2 × CH_3 of cp), 1.74 (6H, s, 2 × CH_3 of cp), 1.90 (6H, s, 2 × CH_3 of cp), 3.00 (1H, s, CC*H*), 3.23 (1H, s, of cp), 6.56 (1H, d, ^3J = 16.57, E-HC=CH), 6.81 (1H, d, ^3J = 16.57, E-HC=CH), 7.16, 7.19, 7.22, 7.25 (4H, m, phenyl)

^{13}C NMR ($CDCl_3$): δ 9.31, 9.85, 11.12, 11.19 (CH_3), 71.31, 79.37, 80.64, 80.81, 81.94, 83.85 (ferrocene, CCH), 122.39, 125.42, 125.76, 128.34, 128.65, 128.92, 129.80, 139.12 (phenyl, C═C).

(*E*)(*Z*)-[2-(3-Iodophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (5). A Schlenk vessel was charged with (3-iodobenzyl)triphenylphosphonium bromide ([36901-75-4], 3.910 g, 7.0 mmol) and 100 mL of anhydrous THF. (3-Iodobenzyl)-triphenylphosphonium bromide was prepared according to the literature.[38] The suspension was cooled to −60 °C (ethanol/liquid nitrogen bath). Afterward, potassium *tert*-butylate (0.900 g, 8.0 mmol, 1.15 mequiv) was added. The cooling bath was removed, and the deep red mixture was stirred for 30 min at 0 °C. Subsequently, formyl-1,1′,2,2′,3,3′,4,4′octamethylferrocene ([128925-12-2, 2.00 g, 6.15 mmol, 0.88 mequiv) was added, and the mixture turned red-brown. After stirring for 2 h at room temperature, the mixture was quenched with 30 mL of water, the THF was removed, and the resulting suspension was extracted with 100 mL of diethyl ether. The ether layer was washed with 50 mL of water. After drying with Na_2SO_4, the ether was evaporated. The crude product was purified by column chromatography (basic alumina, 5% H_2O, Aldrich 5016 A, Brockmann III 1.5 × 30 cm, *n*-hexane). The unconverted aldehyde was recovered (1.442 g, 72%). Yields: *E*-[2-(3-iodophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene, 200 mg (6%); *Z*-[2-(3-iodophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene, 200 mg (6%).

***Z*-[2-(3-Iodophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene.** ^{1}H NMR ($CDCl_3$): δ 1.25, 1.46, 1.54, 1.64 (24H, CH_3), 3.30 (1H, s, of cp), 6.29 (1H, d, ^3J = 9.42, Z olefin proton), 6.45 (1H, d, ^3J = 11.68, Z-HC═CH), 6.86, 7.11, 7.41, 7.50 (4H, m, phenyl). ^{13}C NMR ($CDCl_3$): δ 9.13, 9.79, 10.79, 11.00 (CH_3), 71.49, 79.34, 80.43, 80.73, 81.16 (ferrocene), 127.43, 128.69, 128.90, 129.55,1 35.06, 137.43, 140.65 (phenyl, Z-C═C).

***E*-[2-(3-Iodophenyl)ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene.** ^{1}H NMR ($CDCl_3$): δ 1.63, 1.68, 1.79, 1.94 (CH_3), 3.33 (1H, s), 6.56 (1H, d, ^3J = 16.2, E-HC═CH), 6.83 (1H, d, ^3J = 16.57, E-HC═CH), 6.86, 7.11, 7.41, 7.50 (4H, m, phenyl). ^{13}C NMR ($CDCl_3$): δ 9.32, 9.84, 11.13, 11.19 (CH_3), 71.42, 79.53, 80.83, 81.00, 82.20 (ferrocene), 124.23, 124.63, 128.91, 130.32, 134.32, 134.97, 141.26 (phenyl, Z-C═C). MS (FAB) for $C_{26}H_{31}IFe$ (M = 526.28 g/mol): m/z = 526.0 (100%).

3-[2-(1′,2,2′,3,3′,4,4′,5-Octamethylferrocenyl)ethenyl]-benzaldehyde (6). A Schlenk vessel was charged with (1′,2,2′,3,3′,4,4′,5-octamethylferrocenyl)methyltriphenylphosphonium bromide ([185543-98-0], 2.000 g, 3.01 mmol) and 50 mL of anhydrous THF. The suspension was cooled to −50 °C (ethanol/liquid nitrogen bath). Afterward, potassium *tert*-butylate (0.412 g, 3.67 mmol, 1.2 mequiv) was added. The cooling bath was removed, and the deep red mixture was stirred for 30 min at 0 °C. Subsequently, 3-bromobenzaldehyde ([3132-99-8], 2.030 g, 11.0 mmol, 1.1 mequiv) was added, and the mixture turned light red. After stirring for 1 h at room temperature, the mixture was quenched with 30 mL of water, the THF was removed, and the resulting suspension was extracted with 100 mL of diethyl ether. The ether layer was washed with 50 mL of water and 30 mL of brine. After drying with Na_2SO_4, the ether was evaporated. The crude product was purified by column chromatography (silica, 3 × 15 cm, CH_2Cl_2). Yield: 1.143 g (87%). IR (KBr): 2967 (ν_{st-CH_3}), 2948 (ν_{st-CH_3}), 2901(ν_{st-CH}), 1721 ($\nu_{C=O}$), 1628 ($\nu_{st-C=C}$), 1246 (ν_{C-O}). MS (FAB) for $C_{27}H_{32}FeO$ (M = 428.40 g/mol): m/z = 428.10 (100%).

Investigation of the Type of Insertion for 2. The initiator (10 mg) was added to a well-stirred solution of monomer **2** (1.5 equiv) in C_6D_6 (0.7 mL). The reaction mixture turned deep red immediately and was ready for NMR experiments after 15 min. ^{1}H NMR (C_6D_6): δ 6.65 (d, J = 17.3), 6.61 (d, J = 17.3) (second insertion product), 6.65 (d, J = 17.7), 6.61 (J = 17.7) (first insertion product), 6.12 (s, H_β). No signals for H_α.

Synthesis of Poly(2-*block*-norbornene). A solution of the initiator (2 mg) in benzene (1 mL) was added to a well-stirred solution of **2** (200 mg) in C_6D_6 (5.0 mL). The reaction mixture turned deep red immediately and was stirred for 60 h. For analysis, a small aliquot (50 μL) was withdrawn, and ferrocene carbaldehyde (5 mg) was added. Analytical data for this aliquot after workup (precipitation with pentane): M_w = 64 700, PDI = 1.21, corresponding to a DP of 153. Norbornene (200 mg) was added to the initial reaction mixture, and stirring was continued overnight. Finally, ferrocenecarbaldehyde (10 mg) was added. The polymer was precipitated by adding pentane. M_w = 128 300, PDI = 1.54, corresponding to an average norbornene block size of roughly 680.

Silanization of Silica. Silica materials were dried for 2 h in toluene under argon atmosphere using a Dean−Stark apparatus. Afterward, the toluene was removed and the silica dried in vacuo.

(A) Nucleosil. Nucleosil (25 g) was suspended in dry methylene chloride. Dry triethylamine (2.5 mL) and norborn-2-en-5-yltrichlorosilane (1.4 mL) were added. The reaction mixture was stirred for 2 h at 40 °C. Trimethylchlorosilane (0.7 mL) and dimethyldichlorosilane (0.3 mL) were added, and the mixture was stirred at 40 °C for another 2 h. The silanized Nucleosil was filtered off, washed with methanol and ether, and dried in vacuo. Elemental analysis: 1.95% C (corresponding to a capacity of 0.23 mmol of norbornene/g of Nucleosil).

(B) Micra. The reaction was carried out as described for Nucleosil. Triethylamine (15 mL) and norborn-2-en-5-yltrichlorosilane (10 mL) were added to a suspension of Micra (7.5 g) in dry methylene chloride. Trimethylchlorosilane (3.0 mL) and dimethyldichlorosilane (1.5 mL) were added for end-capping. Separation of the silanized Micra from the liquid part of the reaction mixture was performed via centrifugation (3900 rpm, 5 min). The material was washed with methylene chloride and dried in vacuo. Elemental analysis: 0.40% C (corresponding to a capacity of 47.6 μmol of norbornene/g of Micra).

Poly(ethynylferrocene)-Grafted Silica. The initiator (12 mg, 17 μmol) was added to a well-stirred solution of ethynylferrocene (60 mg, 0.29 mmol) in benzene (5 mL). After 5 min, norborn-2-ene-derivatized silica (300-5, 804 mg) was added, and the mixture was stirred for another 12 h. The dark red grafted support was filtered off and washed with diethyl ether and THF. Finally, the silica was added to a solution of iodine (150 mg, 0.59 mmol) in acetonitrile, and the resulting dark green support was filtered off, washed with diethyl ether, and dried in vacuo. The iodine content was determined by elemental analysis (1.86% I), corresponding to an iron content of 0.05 mmol/g.

Poly([2-[4-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene)-Grafted Silica. This support was prepared as described above using [2-[4-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (200 mg, 0.47 mmol), initiator (8 mg, 11 μmol), and norborn-2-ene-derivatized silica (1.18 g). Oxidation was carried out with iodine (250 mg, 0.98 mmol) in acetonitrile. The iodine content was determined by elemental analysis (1.09% I), corresponding to an iron content of 0.03 mmol/g.

Poly([2-[3-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene)-Grafted Silica. This support was prepared in methylene chloride (6 mL) as described above using [2-[3-(ethynyl)phenyl]ethenyl]-1′,2,2′,3,3′,4,4′,5-octamethylferrocene (70 mg, 0.16 mmol), initiator (5 mg, 7.1 μmol), and norborn-2-ene-derivatized Micra (1.50 g). Oxidation was carried out with iodine (80 mg, 0.31 mmol) in acetonitrile. The iodine content was determined by elemental analysis (0.17% I), corresponding to an iron content of 4.5 μmol/g.

Column Packing and HPLC Procedure. A 300−400 mg sample of the stationary phase was suspended in 4.2 mL of 2-propanol and sonicated for 5−10 min. The resulting slurry was packed into a 60 × 2 mm i.d. steel column using methanol and a pressure of 400 bar. A 30 mL aliquot of methanol and 30−50 mL of water were pumped through the column to pack the column properly. Stock solutions of 100 mM Tris buffer were adjusted to pH 8.0 with hydrochloric acid; those of 100 mM *o*-phosphoric acid were adjusted to pH 7.0 using ammonia. These solutions were diluted to 20 mM with water, 20% acetonitrile. Sodium chloride (1 M) or ammonium sulfate (0.5 M) was added to the eluents as gradient formers. Eluents were degassed in an ultrasonic bath prior to use.

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