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Synthesis of α-substituted iminodiacetate ligands: α-hexadienyl derivatives for the selection of lipoxygenase mimics☆

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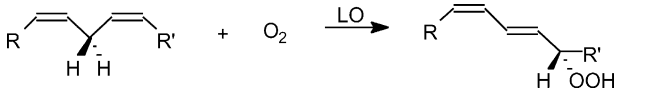
Abstract—Derivatives of iminodiacetic acid (IDA) are important as ligands for metal ions, having numerous applications in separations, sensing, catalysis and medicine. This report describes the preparation of two types of IDA derivatives (**1**, **2**) that could be covalently attached to a polymer or protein surface via a variable length spacer chain. The parent compounds **1** (R′=H) were easily prepared via *N*-alkylation of dimethyl iminodiacetate with esters of 6-bromo-hexanoic acid and subsequent selective ester hydrolysis. Metal complexes of IDA derivatives having an α-dienyl side chain are required for the selection of histidine-rich proteins with potential lipoxygenase activity. The α-hexadienyl side chain of IDA derivative **2** was selectively introduced in the reaction of (2,4-hexadienyl acetate)$Fe(CO)_3$ with a glycine-derived TMS–enol ester. Subsequent demetallation, followed by *N*-carboxymethylation, *N*-deacylation, *N*-alkylation with a trichloroethyl 6-halohexanoate, and TCE–ester cleavage provided the desired α-hexadienyl IDA derivative **2**. Amide formation with IDA acid **1b** demonstrates the feasibility of conjugating the IDA ligands to polymers and proteins while Ni(II)-complexation with the derived IDA triacid **1e** shows the complexing ability of the tethered IDA ligand.

1. Introduction

Metalloenzymes catalyze a variety of important hydrolytic, redox and carbon–carbon bond-forming reactions, some of which have no efficient synthetic counterparts. In order to gain a better understanding of the catalytic mechanisms of metalloenzymes and to develop comparable abiotic catalysts for synthetic utilization, there have been intensive studies of metal complexes that simulate the structural and functional features of metalloenzymes. A common feature of the active site of many metalloenzymes is a bis- or tris-imidazole (from histidine) ligand set.[1] Accordingly, numerous synthetic poly(amine)- and some poly(imidazole)–metal complexes have been investigated as structural and electronic models for these metalloenzymes.[2] A class of poly(histidine)-ligated metalloenzymes of special interest because of their unique and mechanistically intriguing reactions are the lipoxygenases (LO).[3] These iron enzymes catalyze the regio-, stereo- and enantioselective hydroperoxidation of unsaturated fatty acids (Eq. 1).



(1)

The exceptionally high catalytic activity and selectivity of typical enzymes is thought to result in large measure from the substrate-binding and the co-catalytic functionality of the active site's protein environment (as well as transition state stabilization). In an effort to incorporate these features into semi-synthetic metalloenzyme mimics we are investigating various strategies for implanting poly(imidazole)–metal centers in protein matrices. Our initial efforts in this area provided a hybrid esterase protein with high activity by incorporation of a bis(imidazole)-copper cofactor into the combining site of the 38C2 aldolase antibody.[4]

In an approach to poly(imidazole)–metal-proteins that would include a substrate or transition-state binding site at the metal center we are seeking to select metal/substrate-binding proteins from libraries, either directly (by panning) or via immunization/antibody production. For this purpose we envision employing a strongly, but minimally coordinated metal center that can bind effectively to accessible bis/tris-histidine sites on peptides/proteins. Inclusion of a substrate/transition state element situated near to the metal could select/elicit proteins with complementary binding sites. The strong metal binding affinity of iminodiacetate

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Keywords: Iminodiacetate ligands; Catalysis; Lipoxygenase; Regioselective dienylation; (Diene)iron tricarbonyl complexes.

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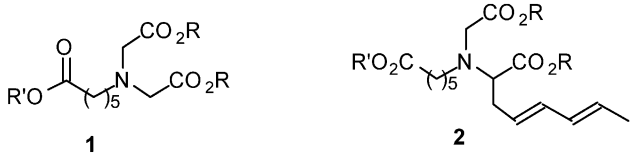
(IDA) ligands[5] and the proven use of M(IDA) complexes for the purification of histidine-tagged proteins by immobilized metal-affinity chromatography (IMAC)[6] has prompted us to seek α-functionalized IDA ligands for the selection of his-rich metal-binding proteins having complementary substrate-binding capability. In the context of our search for LO mimics we have targeted his-phillic IDA derivatives that possess an α-tethered dienyl chain **2** to simulate the putative planar dienyl radical intermediate of the LO enzyme-catalyzed reactions (Fig. 1). In this contribution we describe the first synthesis of such compounds, featuring a new and potentially general method for the α-alkylation of IDA esters.



2. Results and discussion

Two classes of IDA-derivatives were sought, the parent compounds **1** and the α-hexadienyl derivatives **2**, both of which include a nitrogen-linked spacer chain functionalized to enable covalent attachment to a polymer support (for protein binding/selection) or to a protein (for immunization) via amide formation. To allow orthogonal functionalization/deprotection of the three carboxyl functions we used the 2,2,2-trichloroethyl (TCE) ester-derived spacer. The mixed triester **1a** was efficiently synthesized by *N*-alkylation of IDA ester **3** by TCE-6-bromohexanoate **4a**, itself prepared from inexpensive 6-bromohexanoic acid (Fig. 2). Removal of the TCE group of **1a** to afford acid diester **1b** was easily accomplished under standard reducing conditions. To establish the capability of conjugating the *N*-tethered IDA ligands via an amide linkage, the monoacid **1b** was shown to be converted easily to the benzyl amide **1c** using standard amine/carbodiimide methodology. To initially assess the metal-binding ability of the new IDA ligands, the triacid **1e** was prepared by *N*-alkylation of diester **3** to produce triester **1d** and subsequent hydrolysis. The triacid **1e** formed a green nickel complex upon treatment with aqueous $NiCl_2$ which is tentatively formulated as Ni(**1e**-H)$(H_2O)_n$ based on its mass spectrum (ES).

Our original plan for synthesizing the dienyl IDA derivatives **2** was to install the α-dienyl unit via alkylation of an IDA-derived enolate with a simple dienyl electrophile such as sorbyl bromide **5** (Eq. 2). Although seemingly straightforward, in fact, there are very few literature precedents for either the alkylation of IDA ester enolates[7] or for selective nucleophilic substitutions on the bromide **5**.[8] To test this approach, alkylation of the BOC-protected IDA ester **6** was investigated. Treatment of **6** with LDA (THF) followed by benzyl bromide did afford a modest yield of the benzyl derivative **7**. The reported dienyl bromide **5** needed for the synthesis of **8** was produced only in about 80% isomeric purity from the corresponding alcohol under a variety of conditions.[9a,b] Finally, use of bromide **5** (1.2 equiv, 80% purity) in the reaction with the enolate from **6** afforded only 10–15% yield of the alkylated

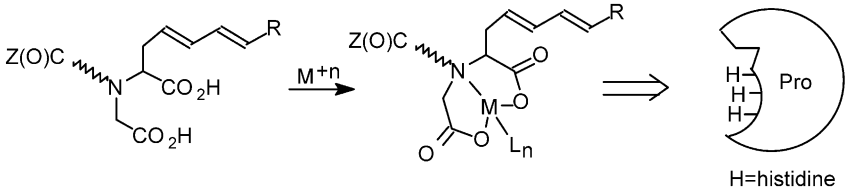


Figure 1.

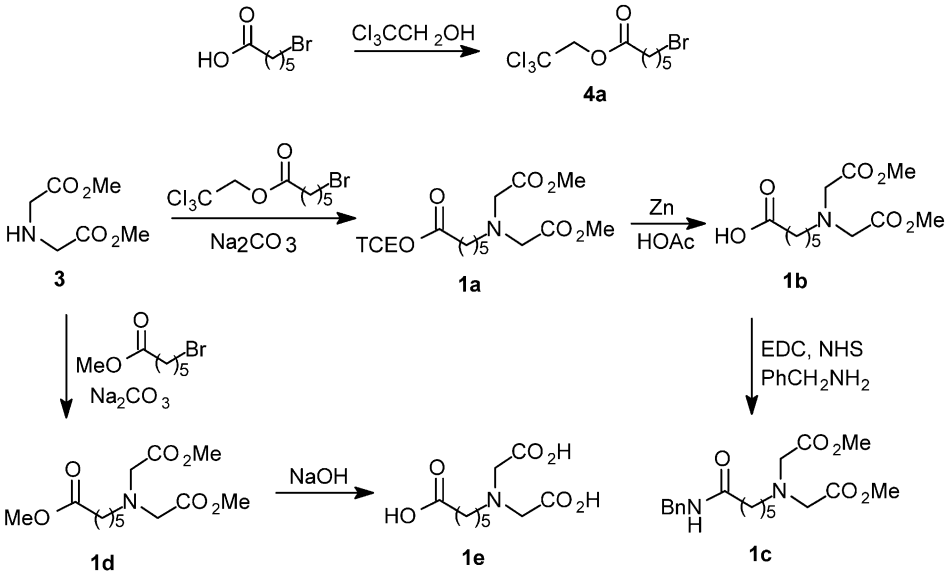
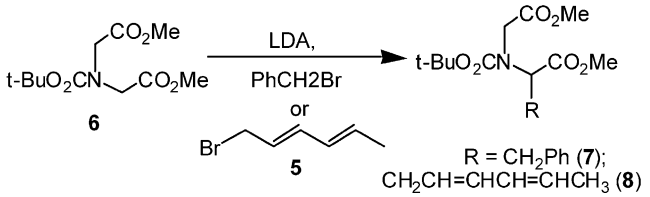


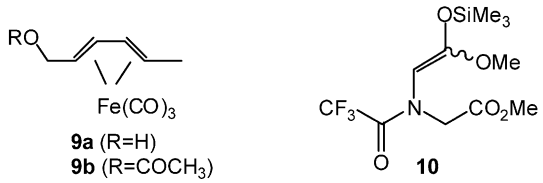
Figure 2.

product(s), which was primarily a non-conjugated regioisomer (rather than **8**) judging by ^{1}H NMR.



(2)

Seeking a more effective and regioselective dienylation method we evaluated an approach based on the electrophilic reactivity of $[(pentadienyl)Fe(CO)_3]^+$ complexes with mild carbon nucleophiles.[10] The readily available (*E*,*E*)-dienyl acetate complex **9b** was selected as the electrophilic component. The initially targeted nucleophilic partner for **9b**, IDA–TMS derivative **10**, proved to be extremely labile and difficult to prepare efficiently. Therefore, we turned to the known glycine derivative **11**[11] as the coupling partner for complex **9b** (Fig. 3). The reaction between **9b** and **11** proceeded readily and regiospecifically at −78 °C in the presence of TMS–OTf to afford the dienyl glycine derivative **12** in excellent yield as a 1:1 diastereomeric mixture (stereounits at the dienyl-iron and α-amino centers). Careful comparison of the ^{1}H NMR spectra of **12** with **9b** and its precursor alcohol **9a** revealed characteristic *trans* coupling constants for protons of the coordinated diene unit (J=8.5 Hz) and highly shielded terminal vinyl protons (0.5–1.2 ppm) in each case, showing that the *E*,*E*-diene sterochemistry is preserved throughout. Demetallation of complex **12** with Ce(IV) cleanly produced the corresponding free dienyl glycine derivative **13** as a single regio- and diastereoisomer (with removal of the stereoinducing –$Fe(CO)_3$ unit). The latter could be converted to the dienyl IDA-derivative **14** upon treatment with methyl bromoacetate under basic conditions.[12] We were pleased to find that the trifluoroacetamide **14** could be selectively deprotected by $NaBH_4$ in methanol (rt, 51%) with no reduction of the ester functions. Attachment of the hexanoate chain by *N*-alkylation of the secondary amine **15** with the TCE bromoester **4a** was found to be very sluggish and inefficient under a variety of conditions.[13] Some improvement was found using the corresponding iodide **4b** (X=I), enabling the preparation of the triester **2a** in moderate yield. The desired acid diester **2b** was obtained when **2a** was treated with excess zinc in glacial acetic acid.[14]



To further demonstrate the synthetic potential of the alkylation of amino acid TMS enol ethers by electrophilic metal complexes, we also examined the reaction between **11** and the dienyl aldehyde complex **16** (Eq. 3). The anticipated product (after demetallation) could be useful for selecting/eliciting metal-binding proteins having a complementary lipoxygenase late transition state/product binding site. In the event, the dienyl alcohol complex **17** was obtained as a partly separable mixture of diastereomers (71% combined), accompanied by a small amount of the dehydrated complex **18** (single isomer, undetermined geometry). These results suggest that the coupling of electrophilic metal complexes with glycine–TMS enol compounds could provide a rather general entry to α-alkylated amino acid and iminodiacetate derivatives.

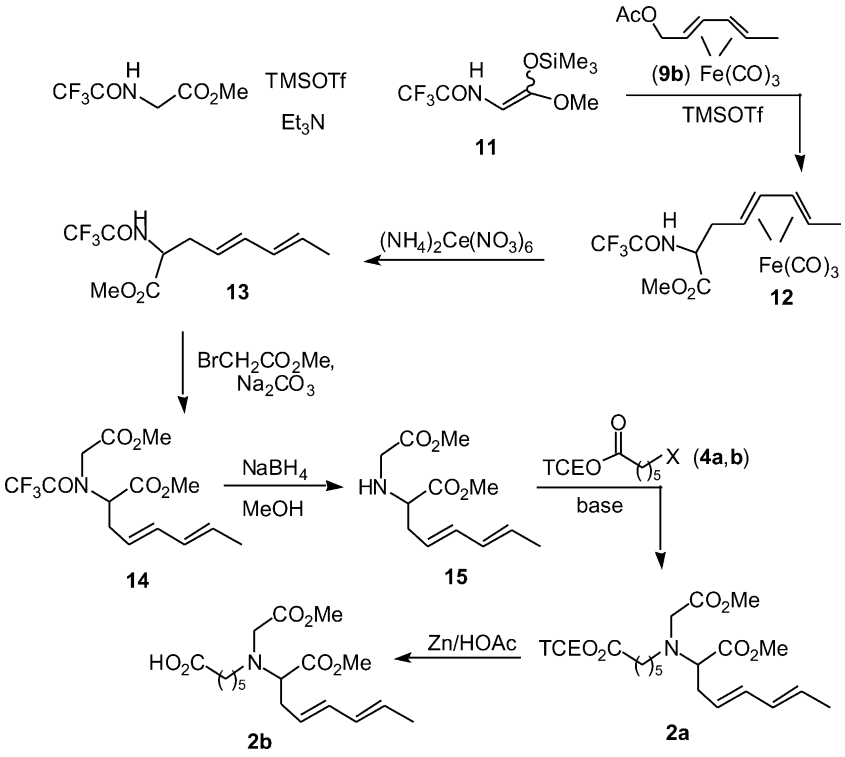
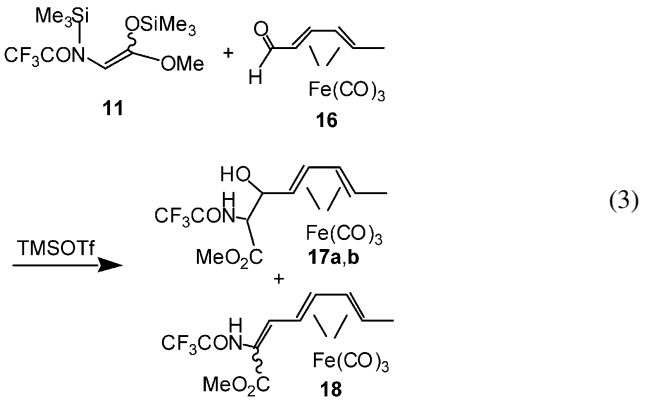


Figure 3.



(3)

3. Summary/conclusions

Preparative methods have been developed to access iminodiacetate derivatives with a functional spacer chain for immobilization and an α-dienyl chain which may be useful for selecting complementary metal binding peptides/proteins with lipoxygenase activity. The α-hexadienyl side chain of IDA derivatives **2** was selectively introduced in the reaction of (2,4-hexadienyl acetate)$Fe(CO)_3$ with a glycine-derived TMS–enol ester. Amide formation with IDA acid **1b** demonstrates the feasibility of conjugating the IDA ligands to polymers and proteins while Ni(II)-complexation with triacid **1e** shows the complexing ability of the tetthered IDA ligand.

Studies of the surface modification and protein conjugation by IDA derivatives **1b** and **2b** are underway. These results, together with investigations of the binding/selection of peptides and proteins by immobilized complexes of the ligands, will be reported in due course.

4. Experimental

4.1. General information

All moisture sensitive reactions were carried out under a dry N_2 atmosphere. All reaction temperatures (°C), except room temperature (rt), correspond to the external bath temperatures.

The following compounds were prepared by reported methods: dimethyl iminodiacetate,[15] dimethyl *N*-*t*-Boc-iminodiacetate,[15] *N*-trifluoroacetyl glycine methyl ester,[16] 2,4-hexadienyl bromide,[9a] (*E*,*E*-2,4-hexadienol)$Fe(CO)_3$,[17] (*E*,*E*-2,4-hexadienyl acetate)$Fe(CO)_3$,[18] (*E*,*E*-2,4-hexadienal)$Fe(CO)_3$[19] and methyl 6-bromohexanoate.[20]

Analytical TLC plates were pre-coated with silica gel 60 F(254). Visualization was accomplished using short wavelength UV light and/or exposing the plate to an iodine chamber. Flash column chromatography (FCC) was performed using 200–400 mesh silica gel with nitrogen pressure. Preparative thin-layer chromatography was performed using Partisil PK6F silica gel plates (Whatman). Triethylamine was dried by distillation from CaH_2 before use. Tetrahydrofuran (THF) and diethyl ether were distilled over Na/benzophenone; dichloromethane and acetonitrile were distilled from CaH_2. Dimethylformamide (DMF) used was purchased as pre-dried. All other reagents/chemicals were obtained commercially and were used without any purification.

^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm, δ) relative to TMS (^1H, ^{13}C) or CF_3Cl (^{19}F). Infra-red spectra were recorded on a FTS 135-BioRad FT-IR spectrometer and reported in wavenumbers (cm^{-1}). Mass spectra are recorded on a Micro-mass (Q-TOF) spectrometer using electrospray time-of -flight (ES-TOF). All new compounds were judged to be >95% pure by NMR and TLC.

4.1.1. TCE bromoester 4a. To a solution of 6-bromohexanoic acid (1.00 g, 5.13 mmol) in 50 mL CCl_4 was added *p*-$TsOH\cdot H_2O$ (1.95 g, 10.3 mmol) and 2,2,2-trichloroethanol (2.46 mL, 25.6 mmol) under N_2 and the reaction mixture was refluxed for 12 h using a Dean–Stark apparatus to remove water. After cooling, 10 mL of water was added and the organic phase was separated. The organic phase was further washed with water (2×10 mL), dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure to afford **4a** as an oil (1.30 g, 78.0%), which was pure enough to use directly in the next reaction.

δ 1.52–1.53 (m, 2H), 1.71–1.73 (m, 2H), 1.85–1.90 (m, 2H), 2.47 (t, J=7.4 Hz, 2H), 3.39 (t, J=6.8 Hz, 2H), 4.72 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1 27.7, 32.5, 33.6, 33.9, 74.1, 95.2, 171.9. IR ($CHCl_3$, cm^{-1}): 1755, 2666, 2946. MS (+ESI): calcd 325 (M); found 348 (M+23), 350, 352. HRMS (+ES): calcd 346.8984 (M^+Na); found 346.8982 (M+Na), 348.8925, 350.8921.

4.1.2. TCE triester 1a. To a solution of **3** (0.085 g, 0.53 mmol) in 5 mL acetonitrile was added anhydrous Na_2CO_3 (10 equiv, 0.560 g, 5.28 mmol), followed by addition of a solution of **4a** (1.5 equiv, 0.257 g, 0.792 mmol) in 1 mL acetonitrile under N_2 and the mixture was vigorously stirred at reflux. After 2 days, the solvent was rotary evaporated and the residual solid was triturated with ethyl acetate (3×50 mL) and the mixture filtered. The filtrate was rotary evaporated to give a gummy material which was purified by flash column chromatography using mixtures of ethyl acetate and hexane as eluant to give **1a** as a colorless oil (0.120 g, 56.1%).

^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (m, 2H), 1.50 (m, 2H), 1.70 (m, 2H), 2.42 (t, J=8 Hz, 2H), 2.69 (t, J=8 Hz, 2H), 3.54 (s, 4H), 3.69 (s, 6H), 4.72 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.7, 26.6, 27.6, 33.9, 51.7, 54.2, 54.9, 73.9, 95.2, 171.8, 172.1. IR ($CHCl_3$, cm^{-1}): 1443, 1744, 2952. MS (+ESI): calcd 405 (M); found: 428 (M+23), 406 (M+1), 408, 410.

4.1.3. IDA acid 1b. To a solution of **1a** (0.116 g, 0.286 mmol) in glacial acetic acid (4 mL) under N_2 was added Zn powder (−100 mesh, 1.86 g, 28.6 mmol) and the mixture was stirred vigorously at rt. After 10 h, 50 mL of ethyl acetate was added and the mixture was filtered through a Celite pad. The residue was washed well with excess ethyl

acetate. The combined washings were concentrated by rotary evaporation and the residual acetic acid was removed under high vacuum at 50–60 °C leaving a white solid. To this material was added 50 mL of ethyl acetate and the solution was washed with saturated aqueous $NaHCO_3$ solution (3×10 mL). The organic phase was separated and the aqueous phase was acidified to pH 4 with conc. HCl and then again extracted with ethyl acetate (3×25 mL). The combined ethyl acetate fractions were dried over anhydrous $MgSO_4$, filtered and rotary evaporated to give **1b** as a gum (0.052 g, 66.1%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (m, 2H), 1.47 (m, 2H), 1.62 (m, 2H), 2.32 (t, J=7 Hz, 2H), 2.69 (t, J=7 Hz, 2H), 3.54 (s, 4H), 3.69 (s, 6H), 9.30 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.5, 26.6, 27.4, 33.9, 51.6, 54.1, 54.7, 171.6, 179.3. IR ($CHCl_3$, cm^{-1}): 1699, 1713, 1716, 1732, 1738, 1742. MS (+ESI): calcd 275 (M); found 276 (M+1), 298 (M+23); (−ESI): 274 (M−1). HRMS (+ESI): calcd 298.1267 (M^++Na); found 298.1245 (M^++Na).

4.1.4. Amide 1c. To a solution of **1b** (0.052 g, 0.189 mmol) in 1 mL dry DMF was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.036 g, 0.188 mmol) and *N*-hydroxysuccinimide (0.022 g, 0.19 mmol) under N_2 at room temperature, and the reaction mixture was stirred for 24 h to make the NHS–ester of **1b**. Then a solution of benzylamine (0.024 g, 0.23 mmol) in 0.5 mL dry DMF was added to the active ester solution and the reaction mixture was stirred for another 8 h at room temperature. DMF was removed under vacuum at 40–50 °C to give a gummy material which was purified by preparative TLC (1:2 ethyl acetate/hexane) to afford **1c** as a gum (0.042 g, 61%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (m, 2H), 1.50 (m, 2H), 1.65 (m, 2H), 2.20 (t, J=8 Hz, 2H), 2.68 (t, J=8 Hz, 2H), 3.51 (s, 4H), 3.67 (s, 6H), 4.41 (d, J=6 Hz, 2H), 4.49 (d, J=6 Hz, 2H), 5.75 (br, s), 5.85 (br, s), 7.23–7.31 (m, 5H). IR ($CHCl_3$, cm^{-1}): 1617, 1674, 1695, 1700, 1743, 2934, 2985, 3054, 3314, 3441. MS (+ESI): calcd: 364; found: 365 (M+1).

4.1.5. IDA triester 1d. To a solution of dimethyl iminodiacetate **3** (0.322 g, 2.00 mmol) in 15 mL of dry acetonitrile under N_2 was added a mixture of methyl 6-bromohexanoate (0.627 g, 3.00 mmol) in 0.5 mL acetonitrile and anhydrous Na_2CO_3 (2.21 g, 20.8 mmol). The reaction mixture was stirred at 80–85 °C for 2 days. Acetonitrile was removed by rotary evaporation and the residue was triturated with ethyl acetate (3×40 mL) and filtered. The combined organic washings were rotary evaporated to give a gummy material which was purified by FCC using a mixture of ethyl acetate and hexane (1:15, 1:10, 1:5) to give the desired **1d** as an oil (0.335 g, 58.0%).

^{1}H NMR (300 MHz, CD_3OD): δ 1.20–1.28 (m, 2H), 1.30–1.40 (m, 2H), 1.45–1.55 (m, 2H), 2.22 (t, J=8 Hz, 2H), 2.57 (t, J=8 Hz, 2H), 3.43 (s, 4H), 3.55 (s, 3H), 3.58 (s, 6H). IR ($CHCl_3$, cm^{-1}): 1265, 1731, 2952. MS (+ESI): calcd 289; found 312 (M+23), 313 (M+1+23), 290 (M+1). HRMS (+ESI): calcd 312.1424 (M^++Na); found 312.1419.

4.1.6. Triacid 1e. To a solution of the triester **1d** (0.20 g, 0.70 mmol) in 1 mL methanol was added 1 mL of 2 N NaOH and the solution was stirred at rt for 5 h. After complete disappearance of the starting material (tlc), the reaction was quenched with 2 N HCl adjusting the pH to 5 at 0–5 °C. Water and methanol were removed by rotary evaporation and the solid obtained was dried under vacuum, triturated with methanol (5×25 mL), and the mixture was filtered. The filtrate was rotary evaporated and vacuum dried to give **1e** as a hygroscopic white solid (0.127 g, 73.4%).

^{1}H NMR (300 MHz, D_2O): δ 1.3–1.4 (m, 2H), 1.55–1.61 (m, 2H), 1.65–1.75 (m, 2H), 2.33 (t, J=8 Hz, 2H), 3.19 (t, J=8 Hz, 2H), 3.73 (s, 4H). ^{13}C NMR (75 MHz, D_2O): 23.4, 23.7, 25.0, 33.6, 55.8, 56.9, 170.2, 178.2. MS (+ESI): calcd 247 (M); found 270 (M+23), 293 (270+23), 317 (293+23+1); (−ESI): found 246 (M−1). HRMS (ES+) calcd 270.0953 (M^++Na); found: 270.0966.

4.1.7. Ni complex of 1e. To a solution of the triacid **1e** (0.100 g, 0.405 mmol) in 1.5 mL of distilled water was added a solution of $NiCl_2\cdot 6H_2O$ (0.096 g, 0.41 mmol) in 0.5 mL of distilled water. The pH of the reaction medium was adjusted to pH 7 by dropwise addition of 1 M NaOH and then the mixture was stirred at room temperature for 4 h, followed by heating at 60–65 °C for another 4 h. After cooling to room temperature, the solution was poured onto a small crystallization plate. After vacuum evaporation of the water at rt and drying under high vacuum, 0.143 g of a light green solid (including NaCl) was obtained. The MS of this material showed a major ion cluster at 326/328 for ^{58,60}Ni(**1e**-H)+Na^+, consistent with a formulation of Ni(**1e**-H)$(H_2O)_n$ (n=0–3).

MS (+ESI): calcd 357 [M(^{58}Ni)]; found 326 [M(^{58}Ni–$3H_2O$+Na)], 328 [M(^{60}Ni–$3H_2O$+Na)]; (−ESI): found 302 (^{58}Ni–$3H_2O$), 304 (^{60}Ni–$3H_2O$). HRMS (+ES): calcd 326.0102 (M^+–$3H_2O$+Na); found 326.0176.

4.1.8. Alkylation of 6 with benzyl bromide and sorbyl bromide. To a solution of diisopropylamine (0.167 mL, 1.19 mmol) in 4.5 mL of anhydrous tetrahydrofuran under N_2 at 0 °C was added *n*-BuLi (0.75 mL, 1.19 mmol) and the reaction mixture was stirred for 20 min. The reaction mixture was then cooled to −78 °C whereupon a solution of **6** (0.300 g, 1.19 mmol) in 1 mL of tetrahydrofuran was added dropwise over a period of 7–10 min. The reaction mixture was then stirred at that temperature for 3 h. Then a solution of benzyl bromide (0.204 g, 1.19 mmol) in 5.4 mL tetrahydrofuran was added dropwise at −78 °C and the mixture was stirred for another 7 h while warming to rt. The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL) and then extracted with ethyl acetate (3×25 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. Purification of the crude material by FCC (ethyl acetate–hexane) gave the benzylated product **7** as a gum (0.191 g, 45.5%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (s, 9H), 3.00 (complex m, 2H), 3.15 (complex m, 2H), 3.64 (s, 6H), 3.73 (d, J=3 Hz, 2H), 4.00 and 4.10 (m, 1H, isomeric mixture), 7.19–7.25 (m, 5H). MS (+ESI): calcd: 351 (M); found: 374 (M+23).

When the same methodology was used for alkylation of **6** with 2,4-hexadienyl bromide **5** (1.2 equiv), a mixture of isomeric dienyl products was obtained in low yield with the 2,4-dienyl isomer **8** as a minor component.

4.1.9. TMS–enol ester 11. To a stirred solution of *N*-trifluoroacetyl glycine methyl ester (0.550 g, 2.97 mmol) in 10 mL of dry ether was added anhydrous Et_3N (3.70 mL, 26.5 mmol) under N_2 at rt and the temperature was lowered to 0 °C. To this stirred solution was added trimethylsilyl trifluoromethanesulfonate (1.10 mL, 6.15 mmol) dropwise. After complete addition the mixture was stirred at rt for 8 h. The ethereal phase was separated carefully from the lower salt phase. The salt phase was further washed with 10 mL of dry ether. The combined organic phase was rotary evaporated. Drying under high vacuum afforded **11** as a light red liquid (0.770 g, 94.9%) that was used for the next step without purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.26 (s, 9H), 0.27 (s, 9H), 3.61 (s, 3H), 5.64 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 0.3, 0.5, 54.6, 88.4, 117.9 (q, $J_{C-F}=203$ Hz), 134.8 (q, $J_{C-F}=29$ Hz), 159.0. ^{19}F NMR (282 MHz, $CDCl_3$): δ −71.2.

4.1.10. (*E*,*E*)-Dienyl complex 12. To a solution of **11** (0.762 g, 2.79 mmol) in dry CH_2Cl_2 (15 mL) at −78 °C was added a solution of **9b** (0.876 g, 2.33 mmol) in 1 mL CH_2Cl_2 under N_2. To this stirred solution was added TMSOTf (84 μL, 20 mol%) and the reaction mixture was stirred while monitoring reaction progress by tlc. After disappearance of **9b**, the reaction was quenched with 2 mL of water and allowed to warm to rt. The mixture was diluted with 25 mL CH_2Cl_2 and the organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent rotovapped. Purification of the crude material by FCC using hexane–ethyl acetate as the eluant afforded **12** as a yellow gum (0.810 g, 85.8%).

^{1}H NMR (300 MHz, $CDCl_3$): (1:1 diastereomeric mixture) δ 0.52–0.58 (m, 0.5H), 0.66–0.72 (m, 0.5H), 1.10–1.17 (m, total 1H), 1.37 (d, $J=6.0$ Hz, 3H), 2.03–2.31 (m, 2H), 3.80 and 3.82 (2s, 3H), 4.57–4.64 (m, 1H), 4.89–4.93 (dd, $J=5.0$, 8.5 Hz, 1H), 4.98–5.24 (m, 1H), 6.95 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.3, 35.9, 36.8, 52.4, 53.3, 53.4, 53.7, 53.8, 58.6, 58.7, 83.4, 83.8, 86.4, 86.5, 116.0 (q, $J_{C-F}=289$ Hz), 157.0 (q, $J_{C-F}=21$ Hz), 170.7, 170.8, 211.9. ^{19}F NMR (282 MHz, $CDCl_3$): δ −75.7, −75.8. IR ($CHCl_3$, cm^{-1}): 1214, 1440, 1719, 1734, 1971, 2044, 2856, 2923, 2958, 3327, 3405. MS (+ESI): calcd 405 (M); found 428 (M^++Na), 429 (M+23+1), 344 (428-3CO), 288.10; MS (−ESI): 404 (M−1). HRMS (+ES): calcd 428.0020 (M^++Na); found 428.0019.

4.1.11. (*E*,*E*)-Dienyl ester 13. To a stirred solution of complex **12** (0.802 g, 1.98 mmol) in 30 mL acetone at −78 °C was added ceric ammonium nitrate (1.63 g, 2.97 mmol) under N_2 and the reaction mixture was slowly allowed to warm to −10 °C. Stirring was continued at this temperature for 5 h whereupon the starting material was consumed (tlc). Acetone was removed by rotary evaporation and the residue was dried under vacuum. Ethyl acetate (75 mL) was added to the residue followed by addition of 20 mL of water. The ethyl acetate phase was separated and the aqueous part was further washed with ethyl acetate (2× 25 mL). The combined organic phase was washed with water (3×10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to give a red gummy material. Purification by FCC using a mixture of hexane/ethyl acetate as the eluant afforded **13** as a gum (0.310 g, 59.1%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72 (d, $J=7.5$ Hz, 3H), 2.60 (m, 2H), 3.78 (s, 3H), 4.61 (m, 1H), 5.30 (m, 1H), 5.62 (m, 1H), 6.00 (m, 2H), 6.80 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.0, 34.7, 52.4, 52.9, 115.6 (q, $J_{C-F}=216$ Hz), 122.1, 130.1, 130.6, 135.7, 156.7 (q, $J_{C-F}=28$ Hz), 170.7. ^{19}F NMR (282 MHz, $CDCl_3$): δ −75.9. IR ($CHCl_3$, cm^{-1}): 1178, 1212, 1696, 1700, 1733, 2361, 3326. MS (+ESI): calcd 265 (M); found 288 (M+23), 266 (M+1). HRMS (+ES): calc. 288.0823 (M^+Na); found 288.0790.

4.1.12. Dienyl IDA ester 14. To a solution of **13** (0.305 g, 1.15 mmol) in 7 mL of dry DMF was added NaH (0.033 g, 1.38 mmol) under N_2 and the mixture was stirred at rt for 10 min. Then a solution of methyl bromoacetate (0.22 mL, 2.3 mmol) in 1 mL dry DMF was added dropwise and the reaction mixture was stirred at 80 °C while monitoring by tlc. After disappearance of the starting materials (12 h), the mixture was cooled to 40 °C and the DMF was removed under reduced pressure. The residue was triturated with ethyl acetate (3×50 mL) and the solution was filtered. The organic phase was rotary evaporated to give a gummy material that was purified by FCC using hexane/ethyl acetate to afford **14** (ca. 1.4:1 amide rotomeric mixture) as a gum (0.190 g, 49.0%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (d, $J=6.6$ Hz, 3H), 2.50–2.70 (m, 2H), 3.69 and 3.70 (2s, 3H), 3.73 (s, 3H), 4.10 and 4.20 (complex m, 2H), 4.62 (m, 1H), 5.30–5.40 (m, 1H), 5.60–5.70 (m, 1H), 5.85–6.10 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.0, 32.2, 33.5, 45.7, 52.4, 52.8, 115.0, 122.8, 130.0, 130.6, 135.2, 157.5, 167.7, 169.4. ^{19}F (282 MHz, $CDCl_3$): δ −68.15, −69.43. IR ($CHCl_3$, cm^{-1}): 1695, 1699, 1749, 2957. MS (+ESI): calcd 337 (M); found 360 (M+23), 338 (M+1), 268 (M−CF_3), 697 [(M×2)+23].

4.1.13. IDA amine 15. To a stirred solution of the *N*-trifluoroacetyl derivative **14** (0.186 g, 0.551 mmol) in 5 mL dry methanol was added $NaBH_4$ (0.052 g, 1.38 mmol) in portions under N_2 at −5 °C. The reaction was stirred at rt while monitoring its progress by tlc. After 5.5 h, the reaction was quenched with glacial acetic acid at 0 °C by lowering the pH to 6. Methanol was rotary evaporated, 50 mL of ethyl acetate was added, and the solution was washed with 20 mL of water. The aqueous phase was further extracted with ethyl acetate (3×25 mL). The combined organic phase was dried over anhydrous $MgSO_4$, filtered and rotary evaporated. Purification of the crude material by column chromatography on silica with ethyl acetate/hexane gave the **15** as a gum (0.068 g, 51.2%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.69 (d, $J=7$ Hz, 3H), 2.30 (br, 1H), 2.40–2.46 (m, 2H), 3.36 (s, 2H), 3.41 (m, 1H), 3.68 (s, 6H), 5.40 (m, 1H), 5.60 (m, 1H), 6.00 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.1, 36.3, 48.9, 51.9, 60.5, 125.1, 128.8, 131.1, 134.1, 172.2, 174.1. IR ($CHCl_3$, cm^{-1}): 1739, 1742, 2953, 3019, 3338. MS (+ESI): calcd 241 (M); found

264 (M+23), 242 (M+1). HRMS (+ESI): calcd 264.1211 (M^++Na); found 264.1232.

4.1.14. 2,2,2-Trichloroethyl-6-iodohexanoate 4b. To a solution of 2,2,2-trichloroethyl-6-bromohexanoate (0.500 g, 1.53 mmol) in 5 mL acetone was added NaI (0.230 g, 1.53 mmol) at room temperature under N_2 and the reaction mixture was stirred for 10 h during which time the starting material was completely consumed (tlc). The solvent was removed by rotary evaporation and the crude material was triturated with CH_2Cl_2 (5×15 mL) and then filtered. The filtrate was concentrated by rotary evaporation and then dried under vacuum to afford the desired iodo compound **4b** as an oil which was spectroscopically pure (0.540 g, 95%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (m, 2H), 1.70 (m, 2H), 1.80 (m, 2H), 2.47 (t, J=7.35 Hz 2H), 3.17 (t, J=6.9 Hz, 2H), 4.73 (s, 2H). IR ($CHCl_3$, cm^{-1}): 801, 1754, 2856, 2929. MS (ESI+): calcd 372 (M^+), 395 (M^++23); found 372.9 (M^++1), 374.9 [(M^++1)+2], 376.9 [(M^++1)+4], 394.9 (M^++23).

4.1.15. IDA TCE ester 2a. To a solution of **15** (0.065 g, 0.27 mmol) in 5 mL of dry acetonitrile was added anhydrous Na_2CO_3 (0.285 g, 2.69 mmol) followed by addition of bromide **4a** (0.13 g, 0.40 mmol) under N_2, and the reaction mixture was stirred at 90 °C while monitoring its progress by tlc. After 3d, the acetonitrile was removed by rotary evaporation, and the residue was triturated with ethyl acetate (3×25 mL) and filtered. Ethyl acetate was removed by rotary evaporation and the residue was purified by preparative thin layer chromatography (1:2 ethyl acetate/hexane) to afford **2a** as a gum (0.037 g, 28%). The corresponding reaction with iodoester **4b** gave **2a** in 38% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40–1.50 (m, overlapping, 4H), 1.60–1.70 (m, 2H), 1.70 (t, J=7 Hz, 3H), 2.30 (m, 2H), 2.40–2.50 (m, overlapping, 4H), 3.40 (m, 1H), 3.66 (s, 6H), 4.05 (t, J=3 Hz, 2H), 4.73 (s, 2H), 5.40–5.60 (m, 2H), 6.00–6.10 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.1, 23.9, 27.6, 32.3, 33.4, 33.7, 36.3, 48.9, 51.92, 60.5, 73.9, 95.0, 125.1, 128.8, 131.1, 134.1, 171.8, 172.2, 174.1. IR ($CHCl_3$, cm^{-1}): 1712, 1730, 1742, 2926. MS (+ESI): calcd 485 (M); found 508 (M+23), 509, 510, 511, 512, 513, 514, 515.

4.1.16. IDA acid 2b. To a solution of **2a** (0.035 g, 0.072 mmol) in 2 mL glacial acetic acid under N_2 was added Zn powder (−100 mesh, 0.47 g, 7.2 mmol) and the reaction mixture was stirred vigorously at rt for 14 h. The mixture was then diluted with 50 mL of ethyl acetate and filtered through a Celite pad. The combined filtrate was concentrated by rotary evaporation and the acetic acid left was removed under high vacuum. To the residue was added 40 mL of ethyl acetate; the solution was then washed with aqueous $NaHCO_3$ solution (3×5 mL). The organic phase was separated and the aqueous phase was acidified to pH 4 with conc. HCl and then again extracted with ethyl acetate (3×30 mL). The combined ethyl acetate phase was dried over $MgSO_4$, filtered and concentrated to give **2b** as a gum after vacuum drying (0.010 g, 39%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39 (m, 2H), 1.55 (m, 2H), 1.65 (t, J=7 Hz, 2H), 1.73 (d, J=7 Hz, 3H), 2.35 (t, J=7 Hz, 2H), 2.51 (m, 2H), 2.73 (t, J=8 Hz, 2H), 3.47 (d, J=6 Hz, 1H), 3.58 (s, 2H), 3.71 and 3.73 (2s, 6H), 5.40–5.50 (m, 1H), 5.60–5.70 (m, 1H), 6.00–6.07 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, low concentration): 18.1, 23.8, 27.6, 29.7, 32.4, 33.5, 35.8, 48.5, 52.2, 60.3, 124.3, 129.3, 130.9, 134.7, 177.7. IR ($CHCl_3$, cm^{-1}): 1731, 1735, 2253. MS (+ESI): calcd: 355 (M); found: 394 (M+K); (− ESI) 354 (M−1).

4.1.17. (*E*,*E*)-Dienyl alcohol complex 17. To a solution of **11** (0.100 g, 0.29 mmol) in 5 mL of dry CH_2Cl_2 under N_2 at −78 °C was added a solution of aldehyde complex **16** (0.045 g, 0.190 mmol) in 1 mL dry CH_2Cl_2 followed by addition of TMSOTf (0.1 mL). The reaction mixture was stirred at this temperature for 2.5 h, whereupon the aldehyde was completely consumed (tlc). The reaction was quenched with 1 mL of water and then extracted with CH_2Cl_2 (3× 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and the solvent was removed by rotary evaporation. Purification of the residue by preparative TLC (1:1 diethyl ether/hexane) afforded triene complex **18** (0.016 g, 20.9%), and dienyl alcohol complexes **17a**,**a′** (0.034 g, 42.5%) and **17b** (0.023 g, 28.7%).

Compound **17a**,**a′**. (1.3:1 diastereomeric mixture) ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75 and 0.95 (m, 1H), 1.25 (m, 1H), 1.41 (d, J=6 Hz, 3H), 1.85 and 2.10 (br s, 1H), 3.78, 3.79 and 3.80 (s, 3H), 3.60 and 3.90 (m, 1H), 4.70 (m, 1H), 5.15 (m, 2H), 7.05 and 7.20 (m, 1H). ^{19}F NMR (282 MHz, $CDCl_3$): δ −75.6, −75.7, −75.8. MS (+ESI): calcd: 421 (M); found: 444 (M+23), 404 (M−OH), 865 (2M+23).

Compound **17b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.78 (m, 1H), 1.23 (m, 1H), 1.40 (d, J=6 Hz, 3H), 2.30 (br s, 1H), 3.78 (s, 3H), 4.20 (m, 1H), 4.69 (d, J=6 Hz, 1H), 5.10 (m, 1H), 5.30 (m, 1H), 7.0 (d, J=15 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 19.1, 53.0, 57.8, 59.2, 73.1, 86.8, 169.0. ^{19}F NMR (282 MHz, $CDCl_3$): δ −75.6. IR ($CHCl_3$, cm^{-1}): 1179, 1215, 1726, 1758, 1974, 2045, 2349, 3343 (br). MS (+ESI): calcd: 421 (M); found: 444 (M+23), 445 (M+1+23), 865 (2M+23), 866 (2M+23+1).

Compound **18**. (single diastereomer) ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (m, 1H), 1.48 (d, J=6 Hz, 3H), 2.85 (m, 1H), 3.74 (s, 3H), 5.20 (m, 2H), 6.40 (d, J=12 Hz, 1H), 7.23 (br, s, 1H). ^{19}F NMR ($CDCl_3$): −75.3, −75.7. MS (+ESI): calcd: 403; found: 426 (M+23), 427 (M+1+23), 404 (M+1), 829 (2M+23).

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