Derivatives of Coenzyme F 430 with a Covalently Attached α-Axial Ligand

Part II[1])

Partial Synthesis of the Five Coenzyme F 430 Tetramethyl Esters and of a Derivative with a Coordinating N^{π}-Methyl-L-histidine Ligand Covalently Attached to the Side Chain at C(3) of F 430 *via* a Peptidic Linker

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Dedicated to Professor *Duilio Arigoni* on the occasion of his 75th birthday

Coenzyme F 430 pentamethyl ester **2** was partially hydrolyzed to a mixture of the five F 430 tetramethyl esters **7**–**11**, which were separated by HPLC and identified by means of a full NMR characterization. The tetramethyl ester with a free COOH group at the side chain at C(3) of F 430 was coupled to the N-terminus of the peptidic spacer–ligand construct **12** selected and studied as described before. The UV/VIS and NMR spectra in CH_2Cl_2/3,3,3-trifluoroethanol 6:1 show that the new derivative, the Ni^{II}(3^3-dehydroxy-8^3,12^2,13^3,18^2-tetra-*O*-methyl-F 430-3^3-yl)-L-prolyl-L-prolyl-N^{π}-methyl-L-histidine methyl ester (**13**), is an intramolecular, pentacoordinate, paramagnetic complex. In the same solvent system, the parent 3^3,8^3,12^2,13^3,18^2-penta-*O*-methyl-F 430 (**2**) is four coordinate and diamagnetic even in the presence of equimolar 1*H*-imidazole. Protonation of the axially coordinating histidine residue of **13** gave the diamagnetic tetracoordinate base-off form, which allowed us to establish the constitution of **13** by NMR.

1. Introduction. – In addition to the selection and synthesis of suitable spacer–ligand constructs as described in *Part I* of this series [1a], our plan to synthesize a derivative of coenzyme F 430 (**1**) with a covalently attached α-axial ligand required F 430 derivatives in which one of the three side chains on the α-side was differentiated from all others. Earlier attempts to obtain selectively functionalized side chains by stoichiometric control of the amidation of free coenzyme F 430 (**1**) had failed and, in our hands, always led to a mixture of pentaamides and unreacted pentaacid [1b]. We, therefore, turned to the opposite approach and tried to find conditions allowing selective partial hydrolysis of coenzyme F 430 pentaesters. To increase the chance of finding selectivity of hydrolysis, additional coenzyme F 430 pentaesters with alcohols of varying steric and electronic demands, namely penta-*O*-benzyl- (**3**), pentakis-*O*-(4-nitrobenzyl)- (**4**), and pentakis-*O*-[2-(trimethylsilyl)ethyl]-F 430 (**5**), were synthesized. To this purpose, the original method for preparation of F 430M (**2**) by acid-catalyzed esterification in MeOH had to be substituted by active-ester coupling methods, which, apart from being more versatile, proved to be slightly more efficient than the classical

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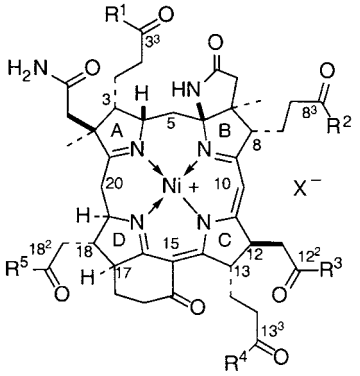
procedure. *Scott* and co-workers have prepared F 430 pentaamides from coenzyme F 430 (**1**) and primary amines using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI)/*N*-(hydroxysulfo)succinimide coupling reagents in aqueous solution but failed to obtain the corresponding dialkylamides with secondary amines under these conditions [2][3]. For esterification rather than amidation of F 430, *Castro*'s reagent (BOP = (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) [4][5] or its nontoxic substitute HATU (= 2-(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) [6–8] with $^{i}Pr_2NEt$/4-(pyrrolidino)pyridine (PP) in DMF proved to be superior to EDCI/*N*,*N*-dimethylpyridin-4-amine (DMAP) and allowed to prepare the pentaesters **3**–**5** in moderate to good yields.



1 Coenzyme F430; $R^1 = R^2 = R^3 = R^4 = R^5 = OH$
2 F430M; $R^1 = R^2 = R^3 = R^4 = R^5 = MeO$, $X = ClO_4$
3 $R^1 = R^2 = R^3 = R^4 = R^5 = PhCH_2O$, $X = ClO_4$
4 $R^1 = R^2 = R^3 = R^4 = R^5 = 4\text{-}NO_2C_6H_4CH_2O$, $X = ClO_4$
5 $R^1 = R^2 = R^3 = R^4 = R^5 = Me_3Si(CH_2)_2O$, $X = ClO_4$
6 $R^1 = R^2 = R^3 = R^4 = R^5 = Me_2N$, $X = ClO_4$
7 $R^2 = R^3 = R^4 = R^5 = MeO$, $R^1 = OH$, $X = ClO_4$
8 $R^1 = R^3 = R^4 = R^5 = MeO$, $R^2 = OH$, $X = ClO_4$
9 $R^1 = R^2 = R^4 = R^5 = MeO$, $R^3 = OH$, $X = ClO_4$
10 $R^1 = R^2 = R^3 = R^5 = MeO$, $R^4 = OH$, $X = ClO_4$
11 $R^1 = R^2 = R^3 = R^4 = MeO$, $R^5 = OH$, $X = ClO_4$

Unfortunately, all attempts to obtain selectivity in the hydrolysis of F 430 pentaesters **3**–**5** under mild conditions such as treatment with iodotetramethylsilane [9], bis(tributyl)tin oxide [10] or, in the case of pentakis-*O*-[2-(trimethylsilyl)ethyl]-F 430 (**5**), with Bu_4NF, failed. In fact, partial hydrolysis of pentamethyl ester F 430M (**2**) with iodotrimethylsilane gave a mixture of tetra- and triesters almost identical in composition to that resulting from aqueous/methanolic acidic hydrolysis as described below.

Although compounds **3**–**5** did not show the selectivity in hydrolysis we had hoped for, reports [3][11] on difficulties in the derivatization of the carboxylic acid side chains of F 430 (**1**) prompt us to describe in the *Exper. Part* the preparation of **3**, as well as that of F 430M (**2**) with the HATU/BOP coupling reagents, besides the inadvertent formation of the first secondary pentaamide derivative **6** of F 430.

2. Partial Hydrolysis of F 430 Pentamethyl Ester and Characterization of the Five Coenzyme F 430 Tetramethyl Esters. – Since we were unable to obtain sufficient selectivity in the hydrolysis of pentaesters, we eventually chose to follow the less than elegant approach of generating a mixture of all five tetramethyl esters by partial hydrolysis of F 430M (**2**) in dilute aqueous/methanolic H_2SO_4 solution and their subsequent separation by HPLC. The optimal temperature and reaction time for obtaining a maximum relative yield of tetraesters and a minimum of more extensively hydrolyzed tri- and diesters were established in preliminary optimization runs with sub-

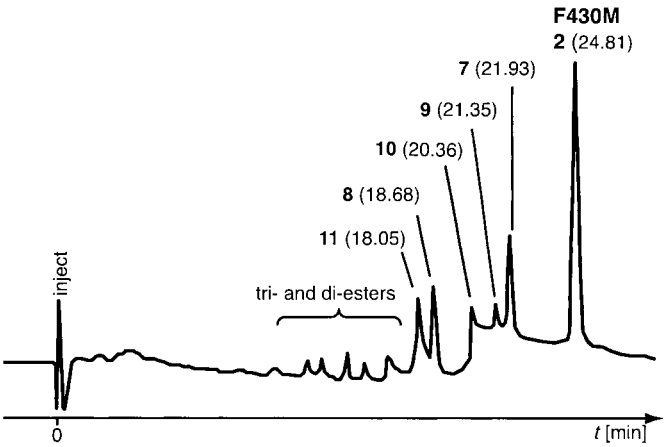
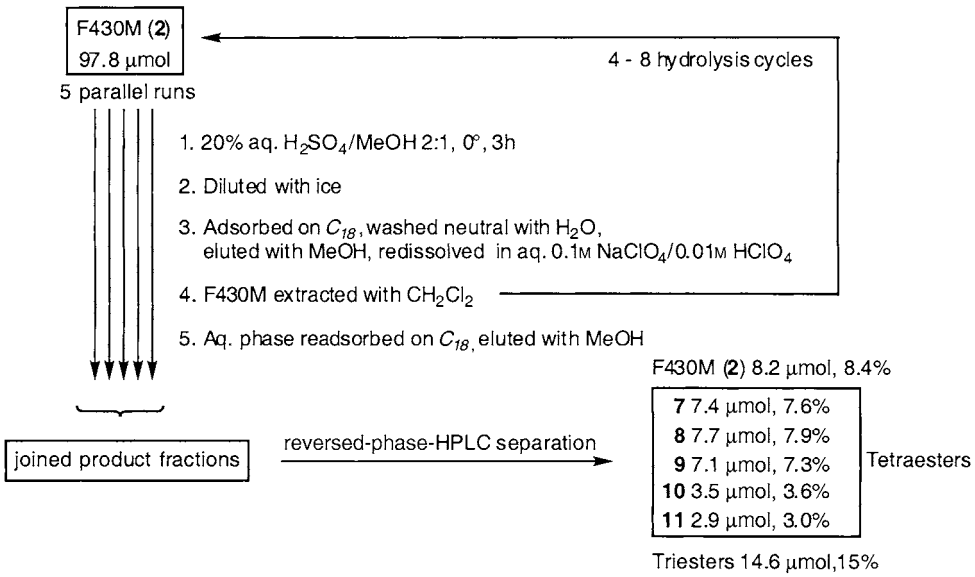


Fig. 1. *Reversed-phase HPLC of the product mixture after partial hydrolysis of F430M* (**2**) *by 20% H_2SO_4 in H_2O/MeOH 2:1* (3 h at 0°). Retention times t_R [min] in parentheses. For HPLC conditions, see *Exper. Part.*

micromolar quantities. *Fig. 1* shows a typical HPLC plot of the product mixture after 3 h of hydrolysis by 20% aq. H_2SO_4/MeOH 2 : 1 at 0°. Then, a larger batch of F 430M (**2**) was hydrolyzed under the optimized conditions with recovery of unreacted **2** and cyclic repetition of the procedure to give a total yield (after HPLC purification) of 30% of the five different tetramethyl esters (*Scheme*).

Initially, it was planned to identify the five tetraesters by re-esterification of the free COOH function with CD_3OH to give *O*-(D_3)methyl-tetra-*O*-methyl-F 430 and

Scheme



comparison of the remaining four ester methyl ^{1}H-NMR signals with those of 3^3,8^3,12^2,13^3,18^2-penta-*O*-methyl-F 430 (= F 430M; **2**), for which the corresponding signals could be assigned unambiguously *via* HMBC. However, this method of assignment proved to be too insecure because of the very small differences in the chemical shifts of the five ester methyl signals and their slight but significant dependence on concentration and solvent composition. Therefore, the identity of each of the five tetramethyl esters had to be established by a complete NMR characterization and localization of the free COOH group by long range ^{1}H,^{13}C correlation (HMBC) of carbonyl C-atoms with the protons of the side chain on the one hand, and – for all but one of the side chains – the ester Me group on the other hand (see *Fig. 2*).

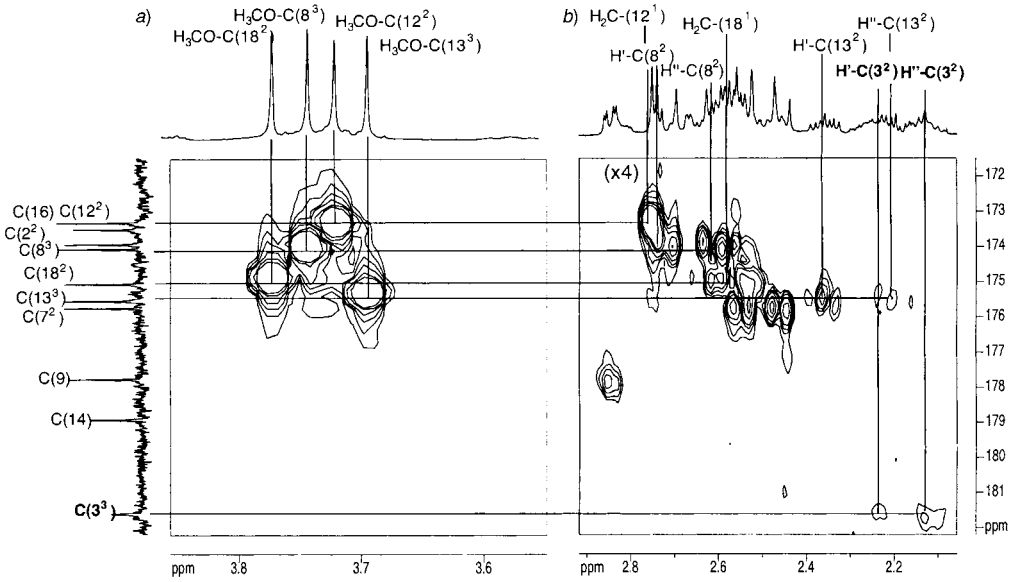


Fig. 2. *Expansions of the ^{1}H,^{13}C-HMBC spectrum of* **7** *in CD_2Cl_2/CF_3CD_2OD 6:1 showing the correlations of the carbonyl C-atoms with* a) *the side-chain protons and* b) *the ester Me groups*

Because Ni^{II} F 430 and its esters have a pronounced tendency to add axial ligands and form penta- and hexacoordinate high spin ($S = 1$) complexes [12–16], high-resolution NMR spectroscopy is possible only in non-nucleophilic, dry solvents. Since the tetramethyl esters were only sparingly soluble in CD_2Cl_2, the solvent in which the structure elucidation of F 430M (**2**) by NMR was originally done [13], it was necessary to add *ca.* 15% (*v*/*v*) of CF_3CD_2OD[3]) to get a sufficiently concentrated but still diamagnetic solution of the tetramethyl esters. The ^{1}H- and ^{13}C-NMR data allowed the identification of the five tetramethyl esters **7**–**11** (see below, *Tables 3* and *4* in the *Exper. Part*).

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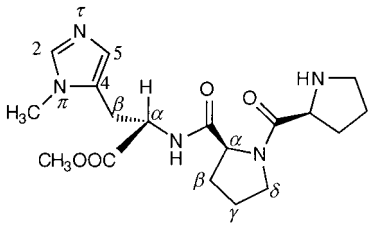
[3]) That coenzyme F430 (**1**) is diamagnetic in 2,2,2-trifluoroethanol was reported for the first time by *Won et al.* [16].



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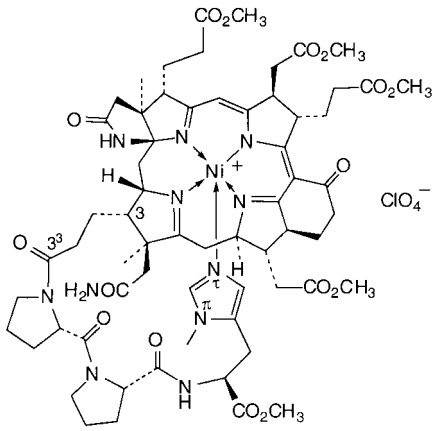

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3. Coupling of 8^3,12^2,13^3,18^2-Tetra-*O*-methyl-F 430 (7) with Pro-Pro-His(π-Me)-OMe (12). – The computational and NMR studies described in *Part I* [1a] resulted in the prediction that tripeptide **12** should be able to assume a conformation leading to intramolecular α-axial coordination of the histidine residue to Ni^{II} if the N-terminal proline was attached to the side chain at C(3) or to that at C(13) of F 430. Since the F 430 tetramethyl ester **7**, with a free COOH group at the end of the side chain at C(3), was one of the major components of the hydrolyzate (26% of the isolated tetraesters), whereas the corresponding 13^3 acid **10** was a minor component (12%), we decided to attach ligand **12** to the free COOH group of **7**. Coupling was achieved with EDCI/DMAP in DMF to give the target molecule **13** in 55% yield (2.6 μmol) after reversed-phase HPLC purification (> 98% pure according to anal. HPLC).

4. Confirmation of Axial Coordination and Generation of a Diamagnetic Base-Off Form by Protonation. – In the solvent mixture CD_2Cl_2/CF_3CD_2OD 6 : 1, in which F 430 pentaesters **2** and **3**–**5** are diamagnetic and give highly resolved NMR spectra, the ^{1}H-NMR spectrum of **13** showed extremely broad, isotropically shifted lines that were at the limit of detectability. The transition from a well-resolved ^{1}H-NMR spectrum to a spectrum with very broad and shifted lines is typically observed when one or more equiv. of a good ligand such as 1*H*-imidazole or 2-methyl-1*H*-imidazole are added to F 430M (**2**) in CD_2Cl_2 and indicates transition from the diamagnetic four-coordinate low-spin form (d^8, $S = 0$) to the paramagnetic (d^8, $S = 1$) five- or six-coordinate high-spin forms. This change of configuration also manifests itself in a subtle change of the UV/VIS spectrum. Although the UV/VIS spectrum of F 430 is dominated by strong π–π^* transitions and the d–d transitions are only detectable by MCD (magnetic circular dichroism) spectroscopy [17][18], the major band at 440 nm broadens and slightly shifts to shorter wavelengths, and the shoulder at the long-wavelength side of the band at 270 nm disappears upon addition of the first axial ligand. In CD_2Cl_2/CF_3CD_2OD 6 : 1, F 430M (**2**, $c = 2$ mM) exhibits UV/VIS spectra of the diamagnetic type even in the presence of 1 equiv. of 2-methyl-1*H*-imidazole because the H-bonding cosolvent CF_3CD_2OD competes with the Ni^{II} center for the ligand. The UV/VIS spectrum of **13**

in this solvent mixture (*Fig. 3*), however, is characteristic for paramagnetic forms as seen by comparison with the corresponding spectra of F 430M in pure, dry CH_2Cl_2 in the presence of 1*H*-imidazole (*Fig. 4*). The fact that the UV/VIS spectrum of **13** is of the paramagnetic type whereas **2** – at the same concentration and in the same solvent system – is diamagnetic even in the presence of 1 equiv. of 2-methyl-1*H*-imidazole, allows us to exclude intermolecular coordination between the histidine of the peptidic spacer and the Ni^{II} center of another molecule of **13**.

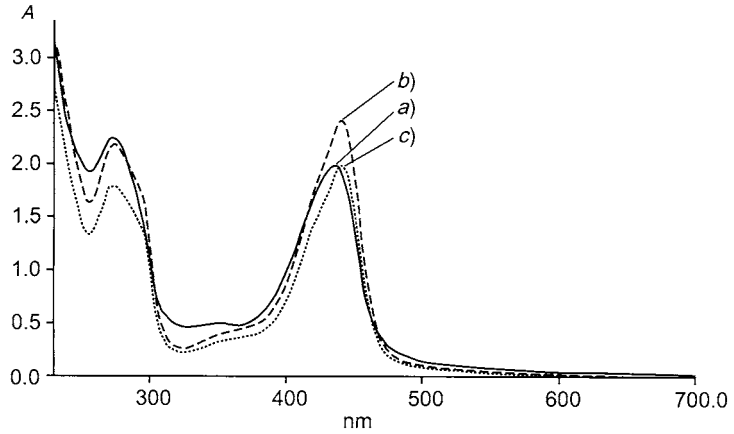

Fig. 3. *UV/VIS Spectra in CH_2Cl_2/CF_3CD_2OD 6:1* ($l = 0.05$ cm) *of* a) *compound* **13** ($c = 1.8$ mM), b) *F 430M* (**2**) ($c = 2.3$ mM), *and* c) *F 430M* (**2**) *in the presence of 1 equiv. of 2-methyl-*^{1}H*-imidazole* ($c = 1.9$ mM)

Because the target compound **13** is paramagnetic, it is impossible to obtain a solution structure or even a confirmation of constitution of the intramolecularly coordinated form by NMR. However, protonation of the coordinating imidazole ring at the N^{τ} atom disrupts axial coordination and generates a base-off form that is tetracoordinate and diamagnetic. Upon addition of CF_3COOD, both the UV/VIS and the NMR spectra changed to the forms typical for diamagnetic F 430 derivatives. This allowed us to record high-resolution NMR data including the two-dimensional spectra required for complete assignment and demonstration of the constitution of **13** (see below, *Tables 5* and *6* in the *Exper. Part*). Interestingly, and in contrast to the free *N*-acylated peptidic spacer–ligand studied in *Part I* [1a] (there compound 14), no slow-exchange processes due to proline *cis*/*trans* amide-bond rotation were detectable in the NMR spectra of N^{τ}-protonated **13**. Hence, the tripeptidic spacer–ligand is present in a single dominant conformation when covalently attached to F 430.

Because, initially, we were not certain whether it would be possible to prove the constitution of the final product by NMR, we considered it mandatory to prepare **13** by the rational sequence of steps described above, namely partial hydrolysis of F 430M (**2**) to the five tetraesters, separation and identification of the tetraesters, and coupling of the linker to a specific tetraester. With target compound **13** and unambiguous spectroscopic confirmation of its constitution in hand, it was of interest whether direct stoichiometric coupling of 1 equiv. of the spacer–ligand to the pentaacid, esterification of the remaining side-chain COOH groups with MeOH and HPLC separation of the

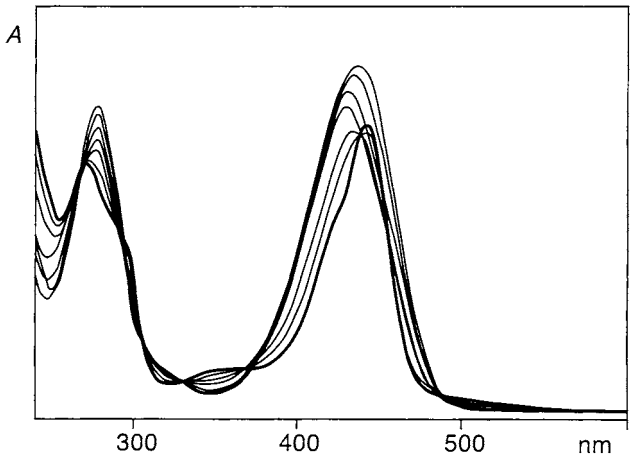

Fig. 4. *UV/VIS Spectra of F 430M* (**2**) *in* CH_2Cl_2 *with increasing concentrations of 1*H-*imidazole*[4]: *transition from the tetracoordinate, low-spin form* (bold face) *to the penta- and hexacoordinate* (last spectrum) *high-spin complexes with axial 1*H-*imidazole ligands.* c(**2**) = $2.9 \cdot 10^{-5}$ M, c(1*H*-imidazole): 0 to 120 mM.

resulting complex mixture would not be more efficient and possibly give higher overall yield of **13** (based on starting F 430 (**1**)) than the stepwise procedure. HPLC-Purified pentaacid **1** in slight excess (5%) was coupled with tripeptide **12** and then esterified with MeOH in a one-pot procedure (for details, see *Exper. Part*). After double HPLC purification, a fraction (> 90% pure) that was identified as **13** by MS and by anal. HPLC (co-injection) was isolated in 8% yield based on F 430. Although the purity of this product was slightly inferior to that of **13** synthesized by the stepwise procedure, the overall yield of this much shorter 'combinatorial' approach was distinctly better than that for the stepwise procedure (*ca.* 3% overall). Of course, this shortcut is only practicable if either a sufficient amount of the target compound can be isolated to ensure full proof of constitution by spectroscopic methods or if the compound has been synthesized first by the stepwise method and can be identified by comparison.

5. Conclusions. – The preparation of the first derivative of coenzyme F 430 with a pending ligand that is constrained by design to coordinate from the α-axial position opens the way towards solution studies of diastereospecifically pentacoordinated and defined mixed hexacoordinated forms of the coenzyme such as they were found by X-ray structure analysis in different states of the enzyme methyl-coenzyme M reductase. Hitherto, studies of the axial coordination at the Ni^{II} center of coenzyme F 430 were hampered by fast ligand exchange that allowed us to observe only equilibrium mixtures of the different axially coordinated forms with exogenous ligands.

The availability of specific pentacoordinated forms may also allow to understand an apparent discrepancy between the axial coordination chemistry of coenzyme F 430 as observed by *Scott* and *Shelnutt* and co-workers on the one hand, and its pentaester derivatives as observed by us on the other hand. Whereas *Scott* and *Shelnutt* interpreted

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[4]) The axial complexation of F 430M (**2**) was studied for the first time by *A. Fässler*, ETH-Zürich [12].

their UV/VIS, MCD, and Resonance-*Raman* data obtained with free coenzyme F 430 (**1**) and exogenous ligands such as H_2O, 1*H*-imidazole, cyanide, and pyridine in terms of an equilibrium between tetracoordinate and hexacoordinate species [15][18–21], we have observed stepwise formation of penta- and hexacoordinate complexes between F 430 pentamethyl ester (**2**) and ligands such as Cl^-, SCN^-, 1*H*-imidazole, pyridine, cyanide, phenolate, thiophenolate, and thiolates in non-coordinating solvents such as CH_2Cl_2 by UV/VIS and NMR[5]) [12][22–24].

As discussed in *Part I* [1a], the axial ligand on the α-side in the enzyme is the carboxamide O-atom of a glutamine residue. Because we could not observe a transition of F 430 pentamethyl ester to the high-spin form in the presence of primary amides in solution [1b], the development of the methodology to prepare intramolecularly coordinating pentacoordinated derivatives of F 430 as described here was carried out with a pending ligand that was known to give high-spin complexes upon axial complexation, namely the 1*H*-imidazole moiety of histidine. The next step, the replacement of the histidine ligand by a suitable glutamine mimic, is currently under investigation in our laboratory. Whether the conformational pre-organization of the proline-containing peptide linker found in the present work will be sufficient to hold a weakly binding ligand such as a carboxamide in the α-axial coordination site of the Ni^{II} center remains to be determined.

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Experimental Part

General. Abbreviations (see also [1a]). EDCI = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, BOP = (1*H*-benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate, HATU = 2-(1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, HPTLC = high-performance thin-layer chromatography, PP = 4-(1-pyrrolidino)pyridine. CH_2Cl_2, toluene, and benzene were distilled over CaH_2 under N_2. MeOH was distilled over Mg under N_2 and was degassed by three freeze-thaw cycles when used for esterifications. THF was distilled over Na-wire/benzophenone under N_2. For the purification of relevant bases and the distillation of DMF (crucial!), see [1a]. Distilled DMF was degassed by three freeze-thaw cycles before being used as a solvent for esterifications. Benzyl alcohol was freshly distilled. Et_3N (*Fluka*) for HPLC was of microselect quality (cut-off $\leq$ 270 nm). *C18* Cartridges (*Sep-Pak*) were washed with 50 ml MeOH or MeCN before they were conditioned with 0.1M $HClO_4$. XAD (*Serva Electrophoresis*; mesh: 0.1–0.2 mm) was extracted with MeOH before use and conditioned with 0.1M $HClO_4$. L-Prolyl-L-prolyl-N^{π}-methyl-L-histidine methyl ester was synthesized as described in [1a]. All other reagents were purchased from *Bachem*, *Novabiochem*, or *Fluka* in the highest available quality and were used without further purification. TLC: for prep. normal-phase TLC, silica gel *60* without fluorescence indicator (*Merck*, 20 × 20 cm); for high-performance TLC (HPTLC), silica gel without fluorescence indicator (*Fluka*, 10 × 20 cm, mesh 2–10 μm); all plates were pretreated, developed (5–6 × CH_2Cl_2/MeOH 13:1), and extracted as described before [13][28]; to eliminate SiO_2 impurities in F 430 samples obtained after this procedure, the extracted product was applied to a small TLC plate (5 × 10 cm), the latter developed (1 × CH_2Cl_2/MeOH 5:1), the product extracted and dissolved in a minimum amount of CH_2Cl_2 (1–1.5 ml), and the soln. washed with H_2O (100–200 μl), filtered over cotton, and evaporated to dryness (concentration plate/water drop procedure). HPLC: solvent systems for ion-pair chromatography: *A* = 0.1M (Et_3NH)(AcO), *B* = MeCN; solvent systems for reversed-phase chromatography of peptidic F 430 derivatives: *A* = H_2O/MeCN 99.99:0.01 + 1‰ CF_3COOH; HPLC solvents were degassed *in vacuo* before use, anal. reversed-phase column, *Nucleosil 50-5 C18AB*, 250 × 4 mm including precolumn (*Macherey-Nagel*); prep. reversed-phase column, *Nucleosil 50-5 C18AB*, 250 × 10 mm including precolumn

[5]) Our NMR studies of paramagnetic penta- and hexacoordinate F430 derivatives will be published separately.

(*Macherey-Nagel*), detection at 430 nm. UV/VIS: *Lambda-20* spectrophotometer (*Perkin-Elmer*), yields and concentrations of F 430 derivatives were determined by spectrophotometry (ε(430 nm) = 22000 in MeOH).

NMR Spectroscopy: Samples were prepared under N_2; for **13**, a *Shigemi* tube (type $CDCl_3$) was used to increase sensitivity; CD_2Cl_2 (*Glaser*; 99.95 atom-% of D) was distilled over CaH_2 and stored at $-20°$ under N_2; typically, diamagnetic NMR samples were obtained after the 'concentration plate/water drop procedure' (see above); if impurities causing paramagnetism were present after this procedure, the F 430 derivative was taken up in a small amount of CH_2Cl_2 (< 1 ml), washed with 0.1M $NaClO_4$/0.01M $HClO_4$ (3 × 1 ml), filtered over cotton, precipitated with benzene, centrifuged, and dried; adding 50–100 µl of CF_3CD_2OD (*Glaser*; 99.5 atom-% of D) to the sample in CD_2Cl_2 gave also diamagnetic spectra; *Bruker DRX-500* at 26.7°; DQF-COSY, HSQC, and HMBC with gradients for coherence pathways selection; atom labels according to the IUBMB-IUPAC convention for peptides [29]; assignments based on HMBC, HSQC, DQF-COSY, and ROESY with the aid of the program SPARKY [30]. ESI-MS: *TSQ 7000* (*Finnigan*).

Purification of F 430 (**1**). Crude F 430 lyophilate [13][25] was a gift from *R. Thauer*, Max-Planck-Institut für Terrestrische Mikrobiologie, Marburg, Germany, and was first desalted to assure better solubility in org. solvents. Thereto, the crude product was dissolved in 0.1M $HClO_4$, brought onto a conditioned XAD column (5 × 2 cm) or on a conditioned *C18* cartridge, washed with H_2O, and eluted with MeOH. The eluate was diluted with THF and evaporated, and the residue was 3× co-evaporated with toluene/THF and dried under h.v. overnight before being used for synthesis. To obtain anal. pure starting material, **1** was purified by reversed-phase ion-pair HPLC [26][27].

3^3,8^3,12^2,13^3,18^2-Penta-O-*methyl-F 430* (**2**; F 430M). a) *By Methanolysis.* F 430M was prepared by methanolysis following the procedure described earlier [13][28].

b) *With Boc as the Coupling Agent (Typical Procedure)*. Under N_2, desalted F 430 lyophilate (25.7 µmol), BOP (457 mg, 1.03 mmol), PP (56 mg, 385 µmol), and $^iPr_2NEt$ (440 µl, 257 µmol) were dissolved in DMF/MeOH 1:1 (2.0 ml; degassed by three freeze-thaw cycles) at 0° and kept for 3 h at 0°. After additional 2 h at 25° (anal. HPLC: reaction complete), mixture was poured into 0.1M $NaClO_4$/0.01M $HClO_4$ (30 ml) and extracted with CH_2Cl_2 (5–6 × 30 ml), the extract filtered over cotton, and evaporated, and the residue (22.9 µmol, 89%) submitted to prep. TLC (8 normal-phase plates, 1 × CH_2Cl_2, 1 × CH_2Cl_2/MeOH 13:1) and the final 'concentration plate/water drop procedure' (see *General*) on a single HPTLC plate (10 × 20 cm, 1 × CH_2Cl_2, 1 × CH_2Cl_2/MeOH 13:1): 14.8 µmol (57%) of **2**.

The yield was higher when HPLC purified F 430 was used, *i.e.*, with F 430 (**1**; 7.0 µmol), BOP (110 µmol, 49 mg), PP (55 µmol, 8 mg), $^iPr_2NEt$ (8.8 mmol, 1.5 ml) in DMF/MeOH 1:1 (2.0 ml), and adjusted amounts of solvents for extraction as described above: 5.4 µmol (77%) of **2**. ^{1}H- and ^{13}C-NMR (CD_2Cl_2 or CD_2Cl_2/CF_3CD_2OD 91.5:8.5): HMBC-based assignments of the ester Me and ester C=O signals reported for the first time here, *Tables 1* and *2*.

3^3,8^3,12^2,13^3,18^2-Tetra-O-*benzyl-F 430* (**3**). Under N_2, desalted F 430 lyophilate (9.7 µmol), HATU (37 mg, 97 µmol), PP (14 mg, 97 µmol), and $^iPr_2NEt$ (1.6 ml, 97 µmol) were dissolved at 0° in 1.6 mM benzyl alcohol in DMF (1.0 ml; degassed by three freeze-thaw cycles). The soln. was kept at 0° for 1 h and for an additional hour at 25° (HPLC: reaction complete). The mixture was poured into 0.1M $NaClO_4$/0.01M $HClO_4$ (40 ml), extracted with CH_2Cl_2 (3 × 80 ml), filtered over cotton and evaporated. The crude product was purified by prep. TLC (5 normal-phase plates, 3 × CH_2Cl_2, 2 × CH_2Cl_2/MeOH 13:1) and subsequently on 1 HPTLC plate ('concentration plate' 10 × 20 cm, 2 × CH_2Cl_2/MeOH 13:1): 5.1 µmol (53%) of **3**. UV/VIS (CH_2Cl_2): 442 (21900), 324 (300), 272 (19600). ^{1}H-NMR (500 MHz, CD_2Cl_2/CF_3CD_2OD 78 :22, *ca.* 5 µmol): 7.41–7.32 (*m*, 25 arom. H); 5.72 (*s*, H–C(10)); 5.14 (*s*, 4 H, PhCH_2); 5.11 (*s*, 2 H, PhCH_2); 5.08 (*s*, 2 H, PhCH_2); 5.07 (*s*, 2 H, PhCH_2); 4.26 (*t*, *J* = 10.1, H–C(4)); 3.84 (*dt*, *J* = 3.1, 9.2, H–C(13)); 3.52 (*m*, H–C(19)); 3.06 (*dt*, *J* = 4.0, 5.2, H–C(12)); 3.01 (*dd*, *J* = 10.0, 17.0, H′–C(20)); 2.91 (*d*, *J* = 10.0, 17.0, H″–C(20)); 2.78–1.86 (*m*, H–C(8), H′–C(2^1), H–C(3), H–C(17), H′–C(12^1), H″–C(12^1), H′–C(17^2), H″–C(2^1), H′–C(18^1), H′–C(8^2), H″–C(17^2), H–C(18), H″–C(18^1), H″–C(8^2), H′–C(7^1), H″–C(7^1), H′–C(3^2), H″–C(3^2), H′–C(13^2), H′–C(8^1), H″–C(13^2), H′–C(17^1), H″–C(8^1), H′–C(13^1)); 1.80 (*d*, *J* = 14.0, H′–C(5)); 1.77–1.53 (*m*, H′–C(3^1), H″–C(17^1), H″–C(13^1), H″–C(3^1)); 1.41 (*t*, *J* = 14.0, H″–C(5)); 1.14 (*s*, Me–C(7)); 1.03 (*s*, Me–C(2)). ESI-MS (+Q; calc. for $C_{77}H_{81}N_6NiO_{13}$, 1357.22): 1356 (100), 1357 (83), 1358 (71), 1359 (43), 1360 (24), 1361 (10), 1362 (5), 1363 (3).

3^3,8^3,12^2,13^3,18^2-Pentadehydroxy-3^3,8^3,12^2,13^3,18^2-pentakis(dimethylamino)-F 430 (**6**). In a prelimary experiment to synthesize 3^3,8^3,12^2,13^3,18^2-penta-*O*-(tert-butyl)-F 430 according to the BOP esterification method, **6** was inadvertently isolated as the only product because the solvent DMF had been distilled over CaH_2 but not over a fractionating column and, therefore, contained an unkown concentration of Me_2NH. Thus, F 430 lyophilate (17.2 µmol of F 430 chromophor), BOP (228 mg, 0.52 mmol), PP (25 mg, 0.17 mmol), $^iPr_2NEt$ (0.17 mmol, 29 µl), and ^{t}BuOH/CH_2Cl_2/DMF 1:1:1.5 (vol-%, 3.5 ml) were stirred for 16 h. Then the mixture

Table 1. *^{1}H-NMR Data* (500 MHz) *and Assignments*[a]) *for F430M* (**2**) *in CD_2Cl_2 and in CD_2Cl_2/CF_3CD_2OD 91.5 : 8.5. c* (**2**) = 8.6 mM; δ(H) in ppm.

	δ(H)			δ(H)	
	CD_2Cl_2	CD_2Cl_2/CF_3CD_2OD 91.5 : 8.5		CD_2Cl_2	CD_2Cl_2/CF_3CD_2OD 91.5 : 8.5
NH–C(7^2)	6.520	exch.	H′–C(18)	n.d.	2.461
NH–C(2^2) (1 H)	6.265	exch.	H′–C(8^2)	2.575	2.569
H–C(10)	5.721	5.731	H″–C(17^2)	2.499	2.531
NH–C(2^2) (1 H)	5.524	exch.	H′–C(3^2)	2.494	2.288
H–C(4)	4.402	4.389	H″–C(8^2)	2.452	2.479
H–C(13)	3.818	3.824	H′–C(18^1)	2.453	2.564
H–C(19)	3.515	3.508	H′–C(7^1)	2.367	2.452
MeO–C(8^3)	3.713	3.712	H″–C(7^1)	2.367	2.383
MeO–C(18^2)	3.693	3.684	H″–C(18^1)	2.367	2.488
Meo–C(12^2)	3.692	3.645	H′–C(13^2)	2.312	2.312
MeO–C(3^3)	3.650 (or 13^3)	3.66	H′–C(8^1)	2.306	2.235
MeO–C(13^3)	3.632 (or 3^3)	3.635	H″–C(3^2)	2.271	2.288
H′–C(20)	3.130	3.080	H″–C(13^2)	2.133	2.148
H–C(12)	3.130	3.072	H″–C(8^1)	1.986	1.966
H–C(8)	n.d.	2.779	H′–C(13^1)	n.d.	1.890
H′–C(2^1)	2.817	2.766	H′–C(5)	n.d.	1.816
H–C(3)	2.752	2.618	H′–C(3^1)	1.783	1.752
H–C(17)	n.d.	2.734	H″–C(17^1)	n.d.	1.774
H′–C(12^1)	2.675	2.754	H″–C(13^1)	n.d.	1.604
H″–C(12^1)	2.675	2.714	H″–C(3^1)	n.d.	1.572
H′–C(17^2)	2.641	2.633	H″–C(5)	n.d.	1.447
H″–C(20)	2.634	3.038	Me–C(7)	1.201	1.170
H″–C(2^1)	2.626	2.576	Me–C(2)	1.117	1.064

[a]) Assignments are based on DQF-COSY, HSQC, and HMBC experiments.

was poured into 0.1M $NaClO_4$/0.01M $HClO_4$ (10 ml), washed with Et_2O (3 × 10 ml), and adsorbed on a conditioned *C18* cartridge. The cartridge was washed neutral with H_2O, the product eluted with MeOH, the soln. evaporated, and the residue submitted to reversed-phase HPLC (A = (Et_3NH)(OAc), B = MeCN); **6** (13.8 μmol, 80%). UV/VIS (CH_2Cl_2): *ca.* 500 (sh), 440 (0.565), 321 (0.100), 273 (0.525). ^{1}H-NMR (500 MHz, CD_2Cl_2/CF_3CD_2OD *ca.* 75 : 25): 5.69 (*m*, H–C(10)); 4.30 (*m*, H–C(4)); 3.76 (*m*, H–C(13)); 3.65 (*m*, H′–C(20)); 3.54 (*m*, H–C(19)); 3.00 (*m*, 1 MeN); 2.99 (*m*, H″–C(20)); 2.99 (*s*, 1 MeN); 2.97 (*s*, 1 MeN); 2.96 (*m*, H–C(12)); 2.94 (*s*, 1 MeN); 2.92 (*s*, 1 MeN); 2.90 (*s*, 1 MeN); 2.89 (*s*, 1 MeN); 2.87 (*s*, 1 MeN); 2.85 (*m*, H′–C(12^1)); 2.84 (*s*, 1 MeN); 2.81 (*s*, 1 MeN); 2.73 (*s*, 1 MeN); 2.73 (*m*, H–C(8)); 2.72 (*m*, H″–C(12^1)); 2.68 (*m*, H–C(17)); 2.68 (*m*, H′–C(2^1)); 2.54 (*m*, H″–C(2^1)); 2.52 (*m*, H′–C(17^2)); 2.50 (*m*, H′–C(18^1)); 2.47 (*m*, H′–C(3^2)); 2.46 (*m*, H–C(3)); 2.45 (*m*, H′–C(8^2)); 2.45 (*m*, H′–C(7^1)); 2.43 (*m*, H″–C(18^1)); 2.40 (*m*, H–C(18)); 2.39 (*m*, H″–C(17^2)); 2.36 (*m*, H″–C(8^2)); 2.35 (*m*, H″–C(7^1)); 2.19 (*m*, H′–C(17^1)); 2.19 (*m*, H′–C(8^1)); 2.16 (*m*, H″–C(3^2)); 1.85 (*m*, H″–C(8^1)); 1.75 (*m*, H′–C(13^1)); 1.71 (*m*, H′–C(3^1)); 1.46 (*m*, H″–C(3^1)); 1.36 (*m*, H″–C(13^1)); 1.71 (*m*, H′–C(13^2)); 1.70 (*m*, H″–C(17^1)); 1.34 (*m*, H″–C(13^2)); 1.33 (*m*, CH_2(5)); 1.12 (*s*, Me–C(7)); 1.00 (*s*, Me–C(2)). ^{13}C-NMR (125 MHz, CD_2Cl_2/CF_3CD_2OD *ca.* 75 : 25): 196.5 (C(17^3)); 187.4 (C(1)); 174.4 (C(9)); 174.1 (C(14)); 173.3 (*C*=O(NMe_2)); 172.6 (C(16)); 172.5, 172.3 (C(2^2), C(7^2)); 172.5, 173.3, 171.6, 170.2 (*C*=O(NMe_2)); 169.4 (C(11)); 97.6 (C(10)); 91.7 (C(6)); 64.5 (C(4)); 63.7 (C(19)); 56.4 (C(8)); 55.6 (C(2)); 50.1 (C(17)); 44.5 (C(18)); 49.8 (C(13)); 48.9 (C(7)); 44.6 (C(12)); 43.1 (C(7^1)); 41.5 (C(2^1)); 39.1 (C(3)); 37.9 (C(12^1)); 37.6 (C(17^2)); 37.3 (MeN); 37.1 (MeN); 36.9 (MeN (3 ×)); 35.6 (C(5)); 35.3 (MeN); 35.2 (MeN (2 ×)); 35.1 (MeN (2 ×)); 34.2 (C(18^1)); 31.5 (C(13^1)); 31.1 (C(3^2)); 30.9 (C(8^2)); 28.1 (C(20)); 25.0 (C(17^1)); 21.8 (C(8^1)); 19.5 (C(3^1)); 30.4 (C(13^2)); 14.5 (Me–C(7)); 19.9 (Me–C(2)). ESI-MS (+ Q; calc. $C_{52}H_{76}N_{11}O_8Ni^+$, 1041.94): 1039 (12), 1040 (8), 1041 (100), 1042 (63), 1043 (61), 1044 (35), 1045 (18), 1046 (7), 1047 (3), 1048 (2), 1049 (1).

Table 2. *^{13}C-NMR Data* (125 MHz) *and Assignments*[a]) *for F 430M* (**2**) *in CD_2Cl_2 and in CD_2Cl_2/CF_3CD_2OD 91.5 : 8.5. $c(2) = 8.6$ mM;* δ(C) in ppm.

	δ(C)			δ(C)	
	CD_2Cl_2	CD_2Cl_2/CF_3CD_2OD 91.5 : 8.5		CD_2Cl_2	CD_2Cl_2/CF_3CD_2OD 91.5 : 8.5
C(17^3)	193.93	196.18	*Me*O–C(13^3)	51.63 (or 3^3)	52.36
C(1)	188.24	188.27	C(17)	n.d.	50.38
C(14)	n.d.	n.d.	C(7)	49.66	49.85
C(9)	n.d.	176.72	C(13)	49.49	49.95
C(13^3)	172.87	174.01	C(18)	44.86	45.07
C(3^3)	172.78	174.07	C(12)	44.66	44.67
C(8^3)	172.48	173.50	C(3)	43.27	43.30
C(18^2)	172.23	172.93	C(7^1)	43.18	43.42
C(2^2)	171.56	172.62	C(2^1)	41.40	41.84
C(12^2)	171.23	172.29	C(12^1)	39.15	39.18
C(7^2)	n.d.	174.13	C(17^2)	38.11	38.06
C(16)	n.d.	172.81	C(18^1)	n.d.	34.67
C(11)	168.93	169.70	C(3^2)	32.22	32.29
C(15)	n.d.	109.16	C(5)	31.44	36.23
C(10)	n.d.	98.37	C(8^2)	31.24	32.46
C(6)	91.25	91.97	C(13^2)	n.d.	31.68
C(4)	n.d.	65.15	C(13^1)	29.52	30.22
C(19)	62.96	63.33	C(20)	n.d.	27.98
C(8)	56.26	56.74	C(17^1)	25.15	25.13
C(2)	54.22	54.48	C(8^1)	21.83	22.07
*Me*O–C(12^2)	51.89	52.56	C(3^1)	19.74	20.11
*Me*O–C(18^2)	51.89	52.47	*Me*–C(2)	19.80	19.96
*Me*O–C(8^3)	51.86	52.54	*Me*–C(7)	15.19	15.18
*Me*O–C(3^3)	51.84 (or 13^3)	52.48			

[a]) Assignments are based on HSQC and HMBC experiments.

Partial Acidic Hydrolysis of F 430M (**2**) (see *Scheme*). In five parallel experiments, **2** (total amount 97.8 μmol (5 × *ca.* 19.6 μmol)) was dissolved at 0° under N_2 in degassed conc. H_2SO_4 soln./H_2O/MeOH 0.4 : 1.6 : 1 (1–2 ml). After 3 h, the mixture was diluted with ice (30 ml) and adsorbed on a *C18* cartridge, which had been conditioned with 0.1M $HClO_4$. The cartridge was washed neutral with H_2O and the F 430 derivatives were eluted with a small amount of MeOH. Then, the eluent was diluted with 0.1M $NaClO_4$/0.01M $HClO_4$ (100 ml) and extracted with CH_2Cl_2 (4 × 100 ml). The combined org. phase was filtered over cotton wool and evaporated and the residue dissolved in conc. H_2SO_4 soln./H_2O/MeOH 0.4 : 1.6 : 1 (1–2 ml) and hydrolyzed as described above. This procedure was used for each parallel experiment. The combined aq. phases of each hydrolysis run were adsorbed on a *C18* cartridge (conditioned with 0.1M $HClO_4$), the cartridge washed neutral with H_2O, and the F 430 monoacids were eluted with MeOH. After finishing all hydrolysis cycles, 8.2 μmol (8.4%) of F 430M (**2**) were recovered. The combined monoacid fractions (59.2 μmol, 60.5%) of all experiments were purified by ion-pair HPLC (A = 0.1M aq. AcO(Et_3NH)(AcO), B = MeCN) to yield *8^3,12^2,13^3,18^2-tetra*-O-*methyl-F 430* (**7**; 7.4 μmol, 11.6%; HPLC purity ≥ 94%), *3^3,12^2,13^3,18^2-tetra*-O-*methyl-F 430* (**8**; 7.7 μmol, 7.9%; HPLC purity ≥ 85%), *3^3,8^3,13^3,18^2-tetra*-O-*methyl-F 430* (**9**; 7.2 μmol, 7.3%; HPLC purity ≥ 75%), *3^3,8^3,12^2,18^2-tetra*-O-*methyl-F 430* (**10**; 3.56 μmol, 3.6%; HPLC purity ≥ 80%), and *3^3,8^3,12^2,13^3-tetra*-O-*methyl-F 430* (**11**; 2.9 μmol, 3.0%; HPLC purity ≥ 85%). UV/VIS (MeOH): **7**: 429 (1.620), 332 (0.248), 275 (1.615), 247 (0.802); **8**[6]): 432 (1.697), 275 (1.590); **9**: 430 (1.579), 274 (1.564); **10**: 434 (0.773), 329 (0.069), 276 (0.727), 251 (0.418); **11**: 430 (0.652), 277

[6]) According to HPLC, UV/VIS and MS analysis, some fractions contained a small contamination by the corresponding 19,20-didehydro- and 12,13-didehydro-tetra-*O*-methyl-F 430 derivatives (for UV/VIS data of didehydro-F 430 derivatives, see [28] [31]).

Table 3. *^{1}H-NMR Spectra* (500 MHz)[a]) *of Tetra*-O-*methyl-F 430* **7**–**11** *in CD_2Cl_2/CF_3CD_2OD*. δ(H) in ppm.

	7 (3^3-OH)	**8** (8^3-OH)	**9** (12^2-OH)	**10** (13^3-OH)	**11** (18^2-OH)
NH–C(7^2)	exch.	7.535	exch.	exch.	7.522
NH_2–C(2^2)	exch.	exch.	exch.	exch.	exch.
H–C(10)	5.837	5.814	5.847	5.795	5.818
H–C(4)	4.442	4.434	4.426	4.423	4.427
H–C(13)	3.851	3.839	3.749	3.907	3.854
MeO–C(3^3)	–	3.706	3.707	3.712	3.693
MeO–C(8^3)	3.742	–	3.749	3.760	3.779
MeO–C(12^2)	3.720	3.672	–	3.663	3.715
MeO–C(13^3)	3.692	3.675	3.664	–	3.733
MeO–C(18^2)	3.773	3.729	3.734	3.731	–
H–C(19)	3.569	3.527	3.530	3.531	3.568
H–C(12)	3.146	3.081	3.026	3.130	3.130
H′–C(20)	3.133	3.051	3.041	3.04	3.135
H″–C(20)	3.028	3.051	3.041	3.04	3.037
H–C(8)	2.848	2.829	2.882	2.816	2.846
H–C(17)	2.821	2.772	2.776	2.836	2.777
H′–C(12^1)	2.753	2.800	2.622	2.819	2.797
H″–C(12^1)	2.753	2.800	2.553	2.819	2.797
H′–C(2^1)	2.715	2.795	2.761	2.783	2.810
H′–C(17^2)	2.687	2.675	2.695	2.624	2.688
H′–C(8^2)	2.621	2.575	2.543	2.600	2.628
H″–C(2^1)	2.611	2.607	2.584	2.594	2.578
H′–C(18^1)	2.579	2.546	2.668	2.547	2.474
H″–C(18^1)	2.579	2.546	2.546	2.499	2.298
H″–C(17^2)	2.577	2.572	2.561	2.624	2.598
H–C(18)	2.564	2.512	2.485	2.469	2.307
H′–C(7^1)	2.547	2.522	2.498	2.514	2.516
H″–C(8^2)	2.530	2.474	2.543	2.507	2.519
H–C(3)	2.468	2.579	2.561	2.588	2.658
H″–C(7^1)	2.459	2.437	2.409	2.479	2.462
H′–C(13^2)	2.359	2.338	2.378	2.099	2.328
H′–C(8^1)	2.263	2.255	2.264	2.264	2.280
H′–C(3^2)	2.233	2.321	2.316	2.320	2.584
H″–C(13^2)	2.197	2.179	2.216	2.099	2.219
H′–C(17^1)	2.145	2.115	2.112	2.103	2.236
H″–C(3^2)	2.117	2.171	2.316	1.773	2.332
H″–C(8^1)	1.992	1.975	1.971	1.987	2.009
H′–C(5)	1.984	1.844	1.821	1.835	1.885
H′–C(13^1)	1.928	1.905	1.893	1.882	1.939
H″–C(17^1)	1.851	1.801	1.785	1.767	1.778
H′–C(3^1)	1.742	1.775	1.787	1.794	1.813
H″–C(13^1)	1.643	1.599	1.564	1.675	1.667
H″–C(3^1)	1.629	1.622	1.615	1.614	1.627
H″–C(5)	1.469	1.458	1.424	1.435	1.471
Me–C(7)	1.221	1.211	1.193	1.203	1.231
Me–C(2)	1.105	1.092	1.087	1.088	1.103

[a]) Assignments are based on DQF-COSY, HSQC, and HMBC experiments.

Table 4. *^{13}C-NMR Spectra* (125 MHz)[a]) *of Tetra*-O-*methyl-F430* **7**–**11** *in CD_2Cl_2/CF_3CD_2OD.* δ(C) in ppm.

	7 (3^3-OH)	**8** (8^3-OH)	**9** (12^2-OH)	**10** (13^3-OH)	**11** (18^2-OH)
C(17^3)	197.97	197.54	197.45	197.73	198.40
C(1)	189.16	188.74	188.16	188.52	189.36
C(3^3)	181.65	175.11	175.12	175.13	175.32
C(14)	178.90	179.03	179.91[b])	179.57[b])	178.52
C(9)	177.85	177.69	177.52	177.24	177.42
C(7^2)	175.74	175.44	175.33	175.33	175.50
C(13^3)	175.50	175.13	175.53	179.94	175.34
C(18^2)	175.03	173.93	173.85	174.21	180.03
C(8^3)	174.04	176.53	174.93	174.58	174.93
C(2^2)	174.00	173.52	173.17	173.35	173.98
C(16)	173.52[b])	173.45[b])	n.d.	171.05[b])	173.25[b])
C(12^2)	173.34	173.26	177.30	173.37	173.33
C(11)	170.05	170.26	171.95	171.12	170.37
C(15)	109.39[b])	109.29[b])	109.15[b])	109.51[b])	109.46[b])
C(10)	99.16	98.90	98.84	98.49	98.50
C(6)	92.96	92.59	92.18	92.31	92.67
C(4)	65.94	65.51	65.38	65.37	65.63
C(19)	64.16	63.80	63.39	63.64	64.63
C(8)	57.32	57.18	56.75	56.94	57.04
C(2)	54.98	54.84	54.65	54.63	54.37
*Me*O–C(3^3)	–	52.77	52.67	52.76	52.51
*Me*O–C(8^3)	52.81	–	52.65	52.78	52.70
*Me*O–C(12^2)	52.91	52.74	–	52.72	52.66
*Me*O–C(13^3)	52.73	52.74	52.45	–	52.65
*Me*O–C(18^2)	52.87	52.77	52.67	52.70	–
C(17)	50.73	50.70	50.56	50.32	50.97
C(13)	50.52	50.36	50.31	50.24	50.18
C(7)	50.21	50.26	50.20	50.16	50.15
C(18)	45.47	45.45	45.33	44.80	46.97
C(12)	45.09	44.99	46.22	44.37	44.95
C(3)	44.39	43.59	43.56	43.45	43.70
C(7^1)	43.70	43.76	43.58	43.77	43.70
C(2^1)	43.07	42.32	42.34	42.35	42.17
C(12^1)	39.93	39.41	42.20	39.15	39.40
C(17^2)	38.41	38.30	38.16	38.20	38.45
C(5)	36.55	36.54	36.59	36.64	36.60
C(3^2)	36.38	32.02	32.41	32.61	32.80
C(18^1)	35.16	35.08	35.13	35.11	32.87
C(8^2)	32.86	33.02	32.77	32.86	32.78
C(13^2)	32.12	32.65	31.96	32.71	32.00
C(13^1)	30.75	30.69	30.36	30.81	30.45
C(20)	28.82	28.27	28.32	28.14	28.64
C(17^1)	25.53	25.46	25.49	25.43	25.27
C(8^1)	22.66	22.63	22.33	22.44	22.45
C(3^1)	22.10	20.47	20.36	20.52	20.68
*Me*C(2)	20.17	20.35	20.25	20.36	19.71
*Me*C(7)	15.20	15.38	15.23	15.38	15.16

[a]) Assignments are based on HSQC and HMBC experiments. [b]) C(14), C(15), and C(16) were identified based on comparison with [28]; the corresponding cross-peaks in the HMBC spectra were not detected.

(0.701). ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. ESI-MS (+Q; calc. for $C_{46}H_{58}N_6NiO_{13}$, 961.69): **7**: 961.6 (56), 962.6 (28), 963.6 (28), 964.6 (12), 965.6 (8), 983.6 (100), 984.6 (56), 985.6 (54), 986.6 (24), 987.6 (12), 988.6 (8), 988.6 (6); **8**: 961.6 (100), 962.6 (52), 963.6 (52), 964.6 (26), 965.6 (13), 966.6 (4), 967.6 (2), 983.5 (77), 984.5 (52), 985.5 (42), 986.5 (20), 987.6 (8), 988.5 (6); **9**: 961.6 (56), 962.6 (41), 963.6 (40), 964.6 (12), 965.6 (6), 983.6 (100), 984.6 (56), 985.6 (52), 986.6 (24), 987.6 (12); **10**: 961.6 (100), 962.6 (56), 963.6 (54), 964.6 (25), 965.6 (14), 983.6 (37), 984.6 (26), 985.6 (22), 986.6 (8); **11**: 961.6 (80), 962.6 (44), 963.6 (44), 964.6 (20), 965.6 (10), 983.6 (100), 984.6 (66), 985.6 (64), 986.6 (28), 987.6 (16).

(3^3-Dehydroxy-8^3,12^2,13^3,18^2-tetra-O-*methyl-F 430-3^3-yl)*-L-*prolyl*-L-*prolyl*-N$^\pi$-*methyl*-L-*histidine Methyl Ester* (**13**). a) *Selective Coupling*. Under N_2, a soln. of **7** (4.75 μmol), L-prolyl-L-prolyl-N$^\pi$-*methyl*-L-histidine methyl ester (**12**; 17.9 mg, 47.5 μmol), EDCI (45.5 mg, 237 μmol), and DMAP (29 mg, 237 μmol) in DMF (1.0 ml) was stirred at 0° for 1 h, diluted with DMF (1.0 ml), and stirred again for 1 h at 25°. Then the solvent was evaporated under h.v., the residue dissolved in 0.1M $NaClO_4$/0.01M $HClO_4$ (5.0 ml), and the soln. adsorbed on a C_{18} cartridge. After washing with 0.1M $NaClO_4$/0.01M $HClO_4$ (50 ml) and H_2O (50 ml), the crude product was eluted with MeOH, the eluate evaporated, and the residue purified by reversed-phase HPLC ($A = H_2O$/MeCN 99:1 + 0.1% CF_3COOH; B = MeCN + 0.1% CF_3COOH; C = MeCN). 2.62 mmol (55%) of **13**. UV/VIS

Table 5. *^{1}H-NMR Spectra* (500 MHz)[a]) *of (3^3-Dehydroxy-8^3,12^2,13^3,18^2-tetra*-O-*methyl-F 430-3^3-yl)*-L-*prolyl*-L-*prolyl*-N$^\pi$-*methyl*-L-*histidine Methyl Ester* (**13**) *in CD_2Cl_2/CF_3CD_2OD 6:1* (+3% CF_3COOD; $CHCl_3$-type *Shigemi* tube; 270 μl). δ(H) in ppm.

	δ(H)		δ(H)
H–C(3.3)	8.384	H″–C(2^1)	2.580
H–C(2.3)	7.280	H″–C(7^1)	2.579
H–C(α.3)	4.826	H–C(17)	2.578
H–C(α.1)	4.621	CH_2C(18^1)	2.576
H–C(4)	4.509	H″–C(8^2)	2.508
H–C(α.2)	4.427	H–C(18)	2.464
H–C(13)	3.857	H′–C(13^2)	2.382
Me(N$^\pi$.3)	3.854	H′–C(3^2)	2.369
Me(O.3)	3.808	H″–C(3^2)	2.325
H′–C(δ.2)	3.756	H′–C(8^1)	2.276
MeO–C(8^3)	3.747	H′–C(β.1)	2.275
MeO–C(18^2)	3.713	H′–C(γ.2)	2.244
MeO–C(13^3)	3.681	H″–C(13^2)	2.241
MeO–C(12^2)	3.667	H′–C(17^1)	2.121
H″–C(δ.2)	3.643	H′–C(β.2)	2.082
H–C(19)	3.626	H′–C(γ.1)	2.015
CH_2(δ.1)	3.528	H″–C(β.2)	1.999
H′–C(β.3)	3.336	H″–C(γ.2)	1.995
H″–C(β.3)	3.100	H″–C(β.1)	1.989
H–C(12)	3.059	H″–C(8^1)	1.977
H–C(20)	3.050	H″–C(γ.1)	1.954
H–C(8)	2.927	H′–C(5)	1.942
H′–C(2^1)	2.887	H′–C(13^1)	1.918
H′–C(12^1)	2.832	H″–C(17^1)	1.866
H′–C(17^2)	2.729	H′–C(3^1)	1.815
H–C(3)	2.700	H″–C(3^1)	1.616
H–C(20)	2.677	H″–C(13^1)	1.556
H″–C(12^1)	2.658	H″–C(5)	1.422
H′–C(7^1)	2.617	MeC(7)	1.204
H′–C(8^2)	2.602	MeC(2)	1.095
H″–C(17^2)	2.587		

[a]) Assignments are based on HMBC, HSQC, DQF/COSY, TOCSY, and ROESY experiments.

Table 6. *^{13}C-NMR Spectra* (125 MHz)[a]) *of (3^3-Dehydroxy-8^3,12^2,13^3,18^2-tetra-*O*-methyl-F 430-3^3-yl)-*L*-prolyl-*L*-prolyl-*N$^\pi$*-methyl-*L*-histidine Methyl Ester* (**13**) *in CD_2Cl_2/CF_3CD_2OD 6:1* (+3% CF_3COOD; $CHCl_3$-type *Shigemi* tube). δ(C) in ppm.

	δ(C)		δ(C)
C(17^3)	197.45	*Me*O–C(18^2)	52.06
C(1)	187.82	C(α.3)	50.55
C(3^3)	178.95	C(17)	50.21
C(14)	n.d.	C(13)	50.20
C(7^2)	n.d.	C(7)	49.79
C(16)	175.53	C(δ.1)	47.74
C(9)	174.78	C(δ.2)	47.68
C(2^2)	174.68	C(18)	45.15
C(13^3)	174.51	C(12)	44.19
C(8^3)	173.96	C(3)	43.06
CO(.2)	173.25	C(7^1)	42.83
CO(.1)	173.15	C(2^1)	41.93
C(18^2)	172.95	C(12^1)	41.34
C(12^2)	172.54	C(17^2)	37.09
C(11)	172.51	C(5)	35.29
CO(.3)	170.20	C(18^1)	34.15
C(3.3)	134.48	Me(*N$^\pi$*.3)	33.35
C(1.3)	130.24	C(8^2)	31.87
C(2.1)	118.40	C(3^2)	31.64
C(15)	n.d.	C(13^2)	31.35
C(10)	n.d.	C(13^1)	30.06
C(6)	92.76	C(β.2)	28.38
C(4)	65.11	C(β.1)	28.38
C(19)	63.31	C(20)	27.70
C(α.2)	60.51	C(β.3)	25.65
C(α.1)	58.59	C(γ.2)	24.55
C(8)	56.97	C(γ.1)	24.21
C(2)	55.04	C(17^1)	23.94
Me(*O*.3)	53.15	C(8^1)	21.55
*Me*O–C(8^3)	52.17	*Me*–C(2)	19.47
*Me*O–C(12^2)	52.16	C(3^1)	18.94
*Me*O–C(13^3)	52.08	*Me*–C(7)	14.25

[a]) Assignments are based on HSQC and HMBC experiments.

(paramagnetic form, CH_2Cl_2/CF_3CD_2OD 6:1; l = 0.05 mm): 435 (1.9783), *ca.* 420 (flat sh), 328 (0.4597), 273 (2.239). ^{1}H- and ^{13}C-NMR: ESI-MS (+Q1); calc. for $C_{64}H_{84}N_{11}NiO_{16}^+$, 1322.13: 1320.5 (100), 1321.5 (79), 1322.5 (72), 1323.5 (42), 1324.5 (22), 1325.5 (8), 1326.5 (5).

b) *Nonselective coupling.* Under N_2, a soln. of HPLC-purified F 430 (**1**; 5.08 µmol), L-prolyl-L-prolyl-*N$^\pi$*-methyl-L-histidine methyl ester (**12**; 1.8 mg, 4.83 µmol), EDCI (49 mg, 254 µmol), and DMAP (31 mg, 254 µmol) in DMF (1.0 ml) was kept for 1 h at 0° and for 1 h at 25° (reversed-phase HPLC: no more **12** detectable). Then, a second portion of each, EDCI (49 mg, 254 µmol) and DMAP (31 mg, 254 µmol), was added, and the soln. was diluted with MeOH (1.0 ml), kept for 1 h at 0° and for 30 min at 25°, and evaporated. The residue was dissolved in a small amount of 0.1M $NaClO_4$/0.01M $HClO_4$ and the soln. adsorbed on a *C18* cartridge, which was subsequently washed with H_2O, CH_2Cl_2, MeOH, and MeOH/CF_3COOH 99:1. The combined methanolic fraction (2.70 µmol, 53%) was evaporated and the residue purified by reversed-phase HPLC (A = H_2O + 0.01% MeCN + 1‰ CF_3COOH; B = MeCN + 1‰ CF_3COOH; C = MeCN) to give 5 fractions (*Fr.*), which were identified by MS and reversed-phase HPLC co-injection with characterized reference samples from previous experiments (*Fr. 3* and *5*). *Fr. 1* (0.59 µmol, 11.7%) was identified as an isomer

of *(tetradehydroxy-tetrakis(dimethylamino)-F 430-X^{y}-yl)*-L-*prolyl*-L-*prolyl*-N$^{\pi}$-*methyl*-L-*histidine methyl ester* ($X = 3$, 8, 12, 13, 18; $y = 2,3$), *Fr. 2* (0.50 μmol, 9.8%) as a mixture of isomers of *(tetradehydroxy-tetrakis(dimethylamino)-F 430-X^{y}-yl)*-L-*prolyl*-L-*prolyl*-N$^{\pi}$-*methyl*-L-*histidine methyl ester* ($X = 3$, 8, 12, 13, 18; $y = 2,3$) and *(tetra*-O-*methyl-F 430-X^{y}-yl)*-L-*prolyl*-L-*prolyl*-N$^{\pi}$-*methyl*-L-*histidine methyl ester* ($X = 3$, 8, 12, 13, 18; $y = 2,3$), *Fr. 3* (0.41 μmol, 8.1%) as **13**, *Fr. 4* (0.23 μmol, 4.6%) as an isomer of *(tetra*-O-*methyl-F 430-X^{y}-yl)*-L-*prolyl*-L-*prolyl*-N$^{\pi}$-*methyl*-L-*histidine methyl ester* ($X = 3$, 8, 12, 13, 18; $y = 2,3$), and *Fr. 5* (0.33 μmol, 06.4%) as F 430M (**2**). UV/VIS (*Fr. 1*, MeOH): *ca.* 560 (sh, flat), 431 (0.2021), 362 (0.0823), *ca.* 320 (sh), 277 (0.2631). UV/VIS (*Fr. 2*, MeOH): *ca.* 560 (flat sh), 432 (0.3655), 355 (0.0766), 275 (0.3654). UV/VIS (*Fr. 3* (**13**), MeOH): 432 (0.3028), 332 (0.0550), 275 (0.2944). UV/VIS (*Fr. 4*, MeOH): 431 (0.1709), 324 (0.0249), 274 (0.1612). ESI-MS (+Q1); calc. for $C_{68}H_{96}N_{15}NiO_{12}^{2+}$, 1374.5; calc. for $C_{64}H_{84}N_{11}NiO_{16}^{2+}$, 1322.1: *Fr. 1:* 1375.4 (100, MH^{+}), 1376.4 (80), 1377.4 (50); *Fr. 2:* 1320.4 (100), 1321.4 (80), 1375.4 (80); *Fr. 3* (**13**): 1320.4 (100); *Fr. 4:* 1320.6 (100).

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