Analysis of Solid-Phase Reactions: Product Identification and Quantification by Use of UV-Chromophore-Containing Dual-Linker Analytical Constructs**

Geoff M. Williams, Robin A. E. Carr, Miles S. Congreve,* Corinne Kay, Stephen C. McKeown, Peter J. Murray, Jan J. Scicinski, and Stephen P. Watson

The development of chemistry directly on solid phase is often hampered by difficulties associated with monitoring the progress of reactions and establishing product purity.[1] One remedial approach has involved introduction of a dual-linker analytical construct comprising a linear sequence of two chemically orthogonal linkers separated by an analytical component.[2, 3] Cleavage at one linker releases only the substrate of interest in a conventional manner, whereas cleavage at the other affords the substrate attached to the analytical unit, which facilitates identification of that product. This principle was further developed in a recently reported construct[3] that was designed to release an analytical fragment composed of an amine group (generated at the point of cleavage), which enhanced detection by electrospray mass spectrometry (MS; a MS sensitizer), and an isotope label which gave rise to a characteristic split-peak pattern in the mass spectrum (a MS splitter).

Herein, we report further enhancement of the construct properties by the incorporation of a UV chromophore (Figure 1). The intention is to provide a means by which components present in a product mixture may not only be identified by MS, but also be quantified quickly, and accurately by measurement at key UV wavelengths. Following validation of this idea, the construct was applied to the synthesis of an otherwise analytically problematic tetrapeptoid.

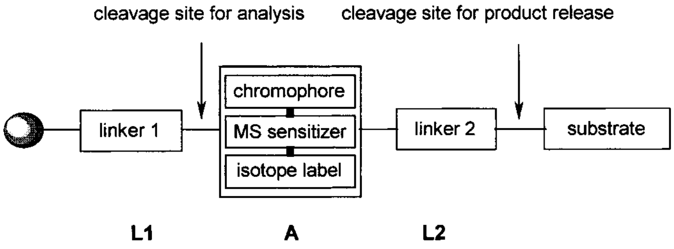
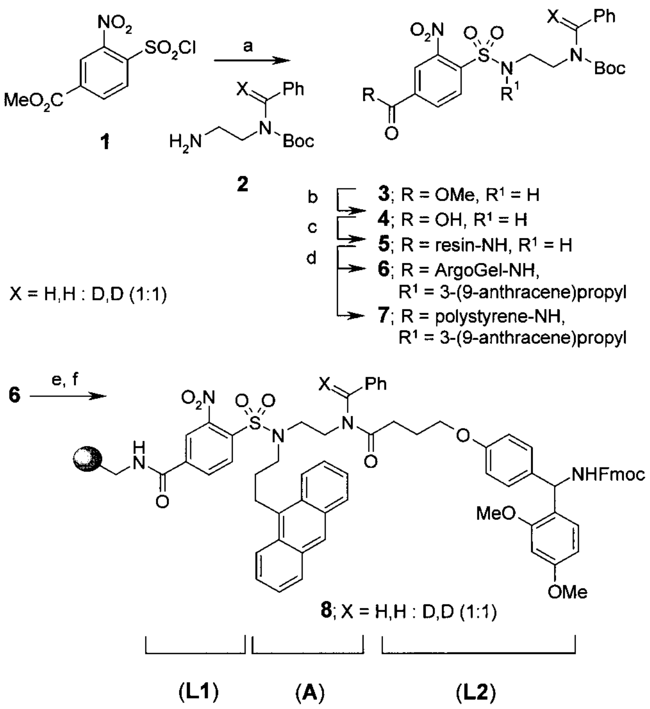


Figure 1. Schematic representation of the analytical construct. Cleavage at linker 2 (**L2**) releases the substrate; alternatively, cleavage at linker 1 (**L1**) releases the substrate bound to an analytical unit (**A**) that enables characterization by HPLC-MS.

In selecting the chromophore a number of factors were considered. Clearly the chromophore must be inert under a wide range of reaction conditions. Furthermore, for quantification purposes the absorption maxima of the UV spectrum must occur in a region generally free of absorbances from other species and also be intense enough to enable detection of material released from a single resin bead. The anthracene moiety, which possesses distinctive and intense absorbances at 254 nm (ε 180000, CH_3CN) and at 386 nm (ε 9000, CH_3CN) appeared to satisfy these criteria.[4]

The synthetic route to the new dual-linker analytical construct **7**[5] is shown in Scheme 1 in which an *o*-nitrophenylsulfonamide group,[6] chosen as linker 1 (**L1**) as this generates the MS sensitizing amine on cleavage, is tethered to the chromophore and the isotope label (**A**). Thus, the sulfonyl chloride **1** and labeled *N*-benzyl-*N*-Boc ethylenediamine (**2**)[7] were combined to give the sulfonamide **3**, the ester group of which was hydrolyzed to the acid **4** and then linked either to aminomethyl ArgoGel (0.4 mmol g^{-1}) or to aminomethyl polystyrene (1.0 mmol g^{-1}) to give the resins **5**. The chromophore was then introduced in quantitative yield by reaction of resins **5** with 3-(9-anthracene)propanol[8] under Fukuyama Mitsunobu conditions[9] to give the ArgoGel resin **6** and the polystyrene resin **7** in >98% purity. A second linker (**L2**,



Scheme 1. a) NEt_3, CH_2Cl_2; b) NaOH, MeOH/dioxane; c) aminomethyl ArgoGel resin (0.4 mmol g^{-1}), PyBOP, HOBt, *i*Pr_2NEt, CH_2Cl_2/DMF; d) 3-(9-anthracene)propanol (5 equiv), PPh_3 (5 equiv), di-*tert*-butyl azodicarboxylate (5 equiv), CH_2Cl_2; e) TFA/CH_2Cl_2, phenol; f) 4-[4-[(2,4-dimethoxyphenyl)[[(9H-fluoren-9-ylmethoxy)carbonyl]amino]methyl]-phenoxy]butanoic acid, PyBOP, HOBt, *i*Pr_2NEt, CH_2Cl_2/DMF. PyBOP = pyridine-benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate; HOBt = 1-hydroxy-1*H*-benzotriazole.

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[*] Dr. M. S. Congreve, Dr. G. M. Williams, Dr. P. J. Murray,[+]
J. J. Scicinski
Glaxo Wellcome-Cambridge Chemistry Laboratory
University Chemical Laboratories
Lensfield Road, Cambridge, CB2 1EW (UK)
Fax: (+44) 1223-331-532
E-mail: mc0067@glaxowellcome.co.uk

Dr. R. A. E. Carr, Dr. C. Kay, Dr. S. C. McKeown, Dr. S. P. Watson
Glaxo Wellcome Medicines Research Centre
Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY (UK)

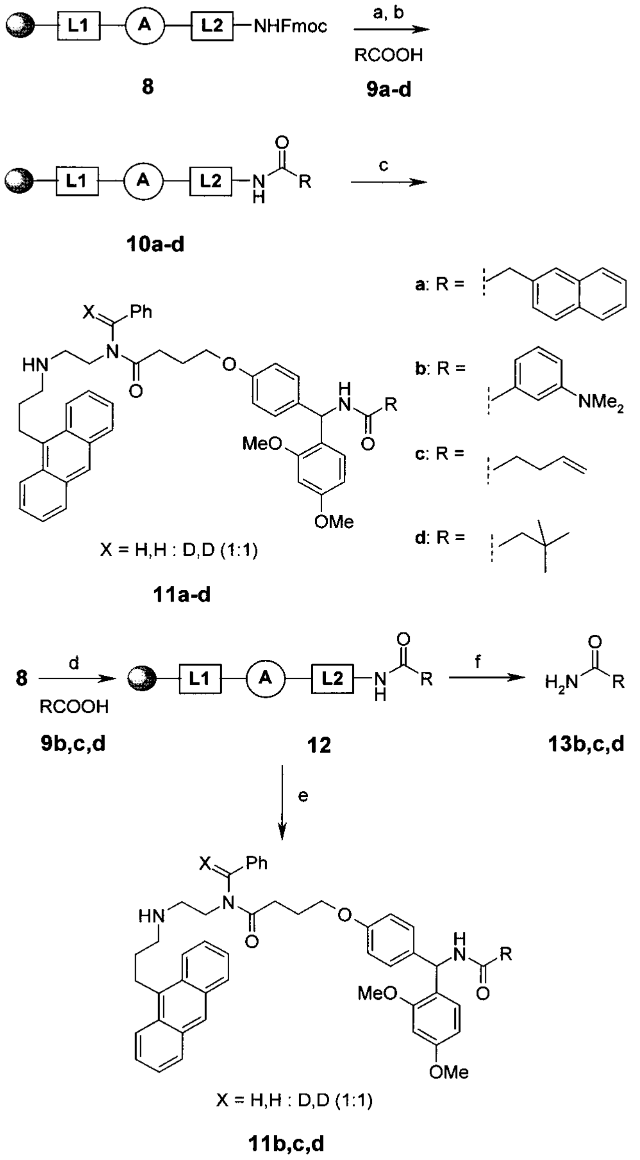
[+] Present address: OSI Pharmaceuticals, 10 Holt Court South, Aston Science Park, Birmingham, B7 4EJ (UK)

[**] We thank Dr. P. Marshall for the provision of analytical data.

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

Scheme 1, a modified Fmoc–Rink linker) was introduced on to the ArgoGel resin **6** to give the complete construct **8** with a purity of >96%.

The effectiveness of the chromophore as a tool for quantification was demonstrated by studying a model mixture of products. The Fmoc protective group of **8** was removed (Scheme 2) and four samples of this resin were derivatized by using the acids **9a–d** to produce the four resins **10a–d**. These acids, which possess a range of UV properties, were carefully chosen. First, on cleavage at **L1** and analysis by gradient HPLC, each of the product amines **11a–d** had a distinctive retention time which enables individual peak areas in the chromatogram of a mixture of **11a–d** to be easily measured. Second, each acid gave rise to at least one resonance signal in the ^{1}H NMR spectrum that was distinct from the others and from the background resonance signals of the construct itself.[10] Thus a correlation could now be made directly between the peak areas of a HPLC chromatogram, observed at the key analytical wavelengths, and the magnitudes of the integration of these resonance signals in the ^{1}H NMR spectrum.



Scheme 2. a) 20% piperidine/CH_2Cl_2; b) **9a**, **b**, **c**, or **d** (4 equiv), PyBOP (8 equiv), HOBt (8 equiv), *i*Pr_2NEt (16 equiv), CH_2Cl_2/DMF; c) 4-mercaptobenzoic acid, *N*-methylmorpholine, CH_2Cl_2; d) **9b** (2 equiv), **9c** (1 equiv), **9d** (3 equiv), PyBOP (8 equiv), HOBt (8 equiv), *i*Pr_2NEt (16 equiv), CH_2Cl_2/DMF; e) 2-mercaptoethanol/DBU, acetonitrile (cleavage conditions A); f) 80% TFA/CH_2Cl_2, phenol. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Table 1 shows the results from the cleavage of a mixture of approximately equal quantities of the resins **10a–d** to release a mixture of **11a**, **11b**, **11c**, and **11d**. The ratio of the components (relative to **11c**) derived from both HPLC peak areas at 254 and 386 nm and from the integration of the ^{1}H NMR spectrum indicate an excellent correlation by using these separate techniques.

Table 1. A comparison of the ratios of components in a four-product mixture as measured by HPLC, relative peak areas at 254 nm and 386 nm, and by ^{1}H NMR spectroscopy.

	254	386	^{1}H NMR[10]
11a	1.30	1.30	1.34
11b	0.87	0.84	0.85
11c	1.00	1.00	1.00
11d	1.00	0.99	1.05

Further validation of the construct was achieved by studying products synthesized as a mixture. A single sample of resin **8** was deprotected and treated (Scheme 2) with a mixture of three acids **9b**, **9c**, and **9d** to afford resin **12**. The ratio of the three components was ascertained by cleavage of a resin sample at **L1** to release a mixture of **11b**, **11c**, and **11d** and by measuring the peak area of each component by HPLC at 254 nm and 386 nm. Determination of the ratio from a single bead of resin in the same way established that the chromophore was suitable for detection at this low level at both wavelengths and that the bulk bead ratio was preserved. Cleavage at **L2** by using trifluoroacetic acid (TFA) released a mixture of the three relatively nonvolatile primary amides **13b**, **13c**, and **13d**. Comparison of the integration magnitudes at the key resonance signals in the ^{1}H NMR spectrum[10] afforded a ratio that correlated well with that determined by HPLC (Table 2).

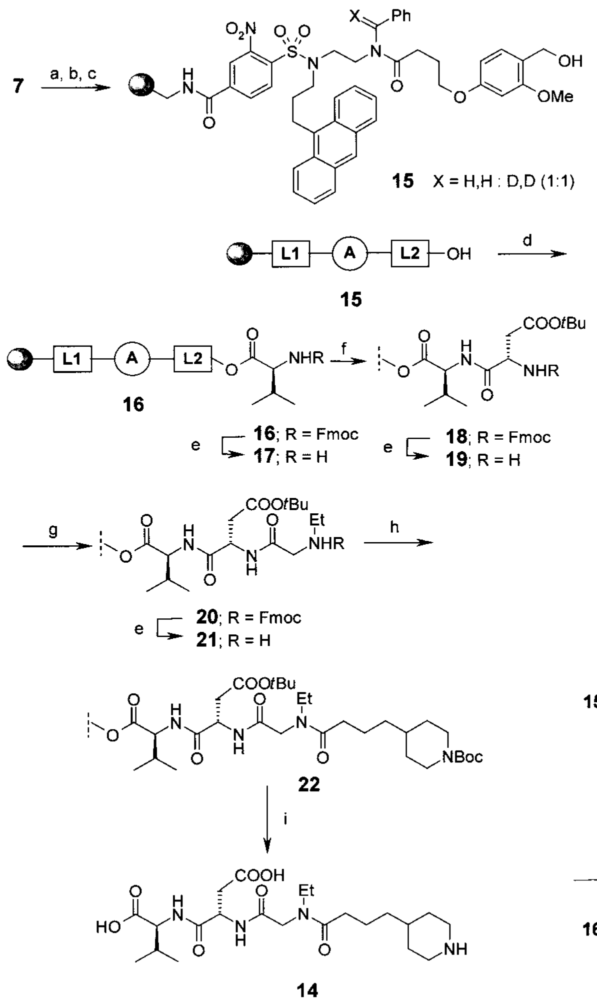
Table 2. A comparison of the ratios of components in a randomly derived three-product mixture as measured by HPLC, by analytical cleavage at **L1**, and by ^{1}H NMR spectroscopy of amide derivatives cleaved conventionally at **L2**.

	254(b)[a]	386(b)[a]	254(s)[a]	386(s)[a]		^{1}H NMR[10]
11b	0.74	0.71	0.71	0.69	**13b**	0.74
11c	1.00	1.00	1.00	1.00	**13c**	1.00
11d	0.44	0.46	0.42	0.41	**13d**	0.42

[a] b = bulk beads, s = single bead; ArgoGel 150 µm beads, theoretical loading 0.29 mmol g^{-1}.

These data show that the anthracene-containing construct can be used to determine accurately the relative quantities of materials in a product mixture.[11]

The utility of the construct was demonstrated in the synthesis of an otherwise analytically problematic target molecule. Owing to the absence of a diagnostic chromophore the fibrinogen receptor antagonist **14**[12] (Scheme 3) presents a



Scheme 3. a) 50% TFA/CH_2Cl_2, phenol; b) 4-(4-formyl-3-methoxyphenoxy)butyric acid (2 equiv), PyBOP (4 equiv), HOBt (4 equiv), iPr_2NEt (8 equiv), CH_2Cl_2/DMF; c) $NaBH_4$, THF; d) FmocVal-OH, PyBOP, HOBt, iPr_2NEt, CH_2Cl_2/DMF; e) 20% piperidine/CH_2Cl_2; f) FmocAsp(O*t*Bu)-OH, PyBOP, HOBt, iPr_2NEt, CH_2Cl_2/DMF; g) Fmoc-*N*-EtGly-OH, PyBOP, HOBt, iPr_2NEt, CH_2Cl_2; h) 4-(4-*N*-Boc-piperidinyl)carboxylic acid, PyBOP, HOBt, iPr_2NEt, CH_2Cl_2/DMF; i) 80% TFA/CH_2Cl_2 (68% overall yield). Fmoc = 9-fluorenylmethoxycarbonyl.

number of synthetic challenges on solid phase, these challenges are: coupling of the hindered Val residue to resin, potentially poor incorporation of Asp, likely generation of a diketopiperazine (DKP), and coupling to a hindered secondary amine.

Beginning with resin **7**, an acid-labile modified Sasrin linker was introduced as **L2**, affording the analytical construct **15** (Scheme 3). The remainder of the synthesis was carried out (Scheme 3), with each step being monitored by the cleavages of a sample of each resin **15**–**22** at **L1** and the analysis of the analytical fragments from HPLC-MS. The chromatograms at each step are reproduced (Figure 2) together with the parent ion in the mass spectrum derived from each major peak. The spectra show the characteristic isotope doublet and confirm the identity of the product in each case. By using this technique each step of the synthesis could be followed through to completion, allowing optimization of reaction conditions as necessary. Critically, the construct enabled the development of conditions for complete loading of the first amino acid on to resin and also provided a means of quantifying the extent of DKP formation during the synthesis of **19**. Reliable quantitative analysis of these processes by conventional methods with small quantities of resin would have been very difficult. Analysis of the resin **22** indicated that the purity of the peptide should exceed 90% on conventional cleavage. This was indeed found to be the case; the purity of the cleaved crude peptide was estimated as approximately 95% by ^{1}H NMR spectroscopy (in 68% overall yield) and was consistent with the literature data for the peptide synthesized in solution.[12, 13]

In conclusion, we have developed an analytical construct that allows monitoring of reactions carried out on solid support and the relative quantification of products, even from a single resin bead, by use of a simple cleavage protocol monitored by standard HPLC-MS analysis.

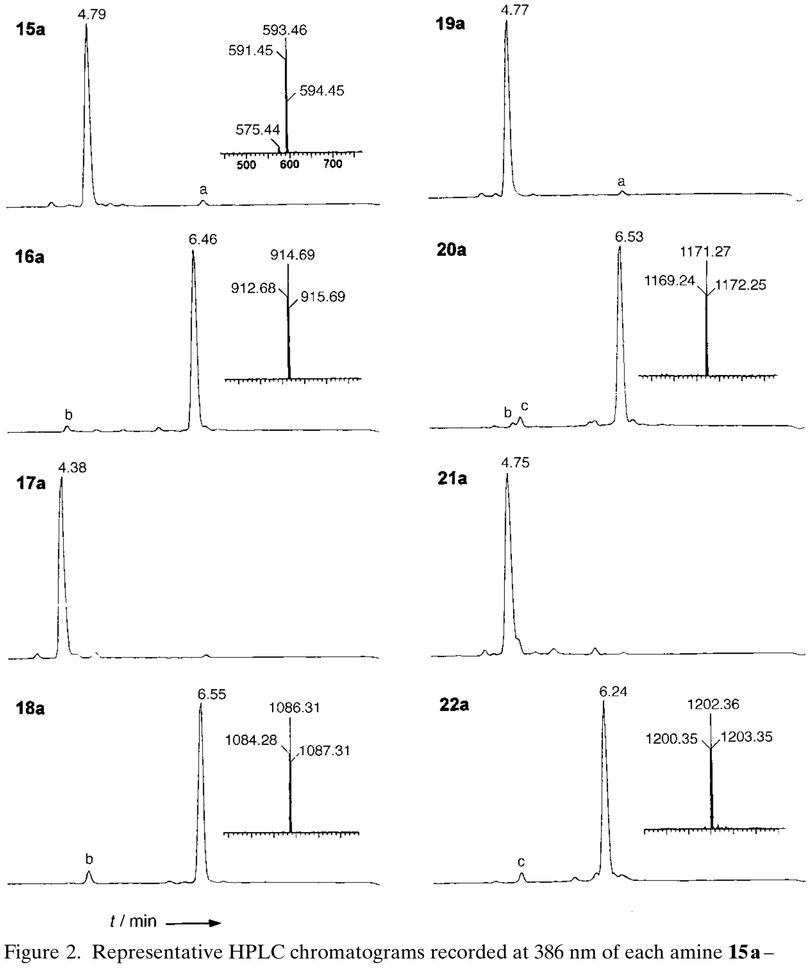


Figure 2. Representative HPLC chromatograms recorded at 386 nm of each amine **15a**–**22a**, obtained by cleavage of the resins **15**–**22** at **L1** (Scheme 3), indicating the progress of each step in the synthesis of **14**. The parent ion of the product in the mass spectrum corresponding to the main peak in selected chromatograms is also given. a: an impurity (2%) occurring after coupling of the second linker. b: indicates a loss (2–3%) of the Fmoc protective group during treatment of **16**, **18**, and **20** with the cleavage medium. c: indicates the reappearance of **15**, indicative of DKP formation in the previous step. Being relatively unreactive, this material is observed in subsequent chromatograms. Approximately 4% of the peptide is lost.

Experimental Section

The purity of materials on the solid support was assessed by cleavage at **L1** and analysis by using high-pressure liquid chromatography (HPLC) on a Hewlett Packard Series 1050 instrument. Column: Supercosil ABZ$^+$PLUS 3.3 cm × 4.6 mm, 3 μm, reverse phase. Eluent A: water, 0.1% trifluoroacetic acid; eluent B: acetonitrile 95%, water 5%, trifluoroacetic acid 0.05%; flow rate: 1 mL min^{-1}; detection: UV (diode array: 215, 230, 254, 386 nm); typical method: gradient 10–95% B in A, over 7 min. Liquid chromatography mass spectra (LCMS) were recorded on a Micromass Platform by using thermospray positive and negative ionization. Column: Supercosil ABZ$^+$PLUS 3.3 cm × 4.6 mm, 3 μm, reverse phase. Eluent A: 10 mM solution of ammonium acetate in water, 0.1% formic acid; eluent B: acetonitrile 95%, water 5%, formic acid 0.05%; flow rate: 1 mL min^{-1}; detection: UV (diode array: 215, 230, 254 nm); typical method: gradient 0–100% B in A, over 3.5 min, 100% B 3.5 min.

Cleavage at **L1** was carried as follows: A) Modified Rink constructs **8**–**12**. A small resin sample was incubated for 5–10 min at room temperature with a solution (10 μL) composed of 2-mercaptoethanol (21 μL), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (70 μL) in acetonitrile (250 μL). After filtration the filtrate was diluted to a final volume of 20 μL with acetonitrile prior to analysis. B) Modified Sasrin constructs **15**–**22**. A small resin sample was incubated for 5–10 min at room temperature with a solution (10 μL) composed of thiophenol (25 μL), and diisopropylethylamine (50 μL) in acetonitrile (250 μL). After filtration the filtrate was evaporated under a stream of nitrogen and the residue dissolved in acetonitrile (20 μL) prior to analysis. Conditions B were chosen to avoid cleavage of the ester bond in compounds **16**–**22**.

Received: April 12, 2000 [Z14983]

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[10] (CDCl$_3$): $\delta = 7.7$ (m, 4H) for **11a**; 5.7 and 4.9 (m, 3H) for **11b**; 2.9 (s, 6H) for **11c**; 0.9 (s, 9H) for **11d** .

[11] In addition by construction of a standard curve using 9-methylanthracene and its use as an internal standard, the absolute quantification of the resin-bound products can be assessed. See Supporting Information.

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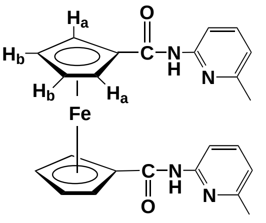
[13] Careful examination of the mass spectral data of each intermediate analytical fragment indicated that no unexpected ion doublets were present, suggesting that the anthracene group was stable to all of the reaction conditions for the synthesis.

Redox-Switched Control of Binding Strength in Hydrogen-Bonded Metallocene Complexes

Jonathan D. Carr, Simon J. Coles,
Michael B. Hursthouse, Mark E. Light,
James H. R. Tucker,* and Joanna Westwood

Redox-switched binding of organic molecules by either organic[1] or inorganic[2] electroactive species is attracting continued interest in the area of supramolecular electrochemistry. Those examples in which the binding interaction is through hydrogen bonds are relevant to recent studies which indicate that hydrogen bonds play an important role in tuning the redox potential of certain proteins.[3] Herein we compare the dicarboxylic acid binding properties of **1** with those of its charged cobaltocenium analogue **2**[4] and show that the strength of the hydrogen bonding interaction can be controlled by changing the oxidation state of the metal in each receptor.



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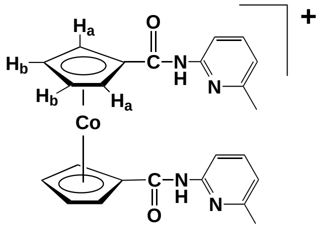

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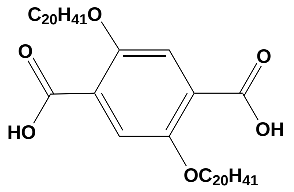

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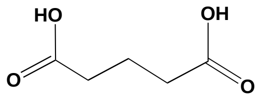

GA

As found previously with **1**[5] and with other organic[6] and inorganic[7] bis-amidopyridine receptors, **2** binds dicarboxylic acids in organic solvents. ^{1}H NMR studies in CDCl$_3$/[D$_6$]DMSO (0.5%) revealed downfield shifts in the amide and Cp proton resonances of **2** upon addition of excess glutaric acid (GA). A titration of the H$_a$ proton resonance against molar equivalents of GA revealed a complex stoi-

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[*] Dr. J. H. R. Tucker, J. D. Carr, J. Westwood
School of Chemistry
University of Exeter
Stocker Road, Exeter, EX4 4QD (UK)
Fax: (+44) 1392-263-434
E-mail: j.h.r.tucker@exeter.ac.uk

Dr. S. J. Coles, Prof. M. B. Hursthouse, Dr. M. E. Light
Department of Chemistry
University of Southampton
Highfield, Southampton, SO17 1BJ (UK)

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