The 3-*N*-phenyl amide of *all-cis*-cyclopentane-1,2,3,4-tetracarboxylic acid as a potential pH-sensitive amine-releasing prodrug; intervention of imide formation around neutral pH

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Dedicated to Professor Jim Coxon on the occasion of his 65th birthday

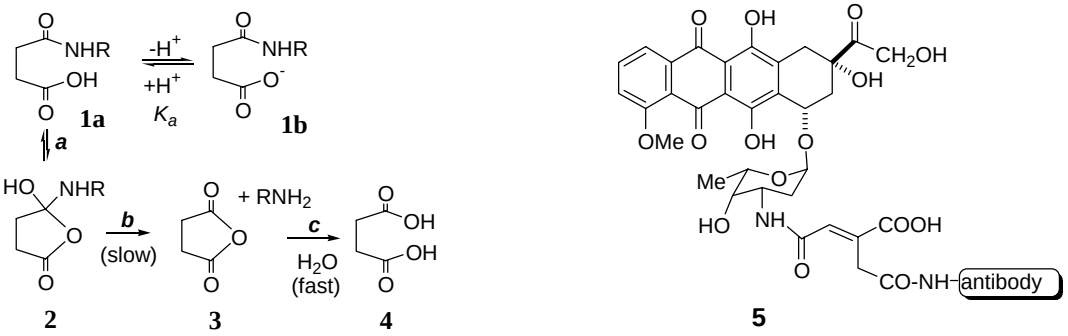
Abstract

To assess the potential utility of the new amide [(1*S*,2*R*,3*R*,4*R*)-3-(phenylamino-carbonyl)cyclopentane-1,2,4-tricarboxylic acid] (**10**) as a pH-sensitive amine-releasing prodrug aimed at selective activation at the low extracellular pH of solid tumours, its reactivity in D_2O was studied between pD 5 and 6.5. Neighbouring group catalysis of amide hydrolysis by un-ionised carboxylic acid groups was expected up to neutral pH, with this decreasing with increasing levels of ionisation. Rate measurements on **10** in D_2O by UV at 27 ^oC gave k_1 7.3 x 10^{-5} s^{-1} (half-life *ca* 2.5 h) at pD 5.02 and 3.0 x 10^{-5} s^{-1} (half-life *ca* 6.5 h) at pD 6.53. However, NMR monitoring of **10** in D_2O showed that at pD 5 and above, formation of the 2,3- and 3,4-*N*-phenylimides of cyclopentane-1,2,3,4-tetracarboxylic acid (**13** and **16**) unexpectedly predominates over amide hydrolysis, thwarting the potential prodrug application. The rates together with variations in the product ratios with pD show that the rate of formation of **13** from **10** is only marginally reduced between pD 5 and 6.5 while the rates of formation of **16** and of hydrolysis products both decrease sharply. We report syntheses of **10** and the new imide **16**, X-ray crystal structure determinations of imides **13** and **16**, as well as NMR data for their solutions in D_2O at different levels of ionization.

Keywords: Amide, imide, neighbouring carboxylic acid group, hydrolysis, mechanism, crystal structure

Introduction

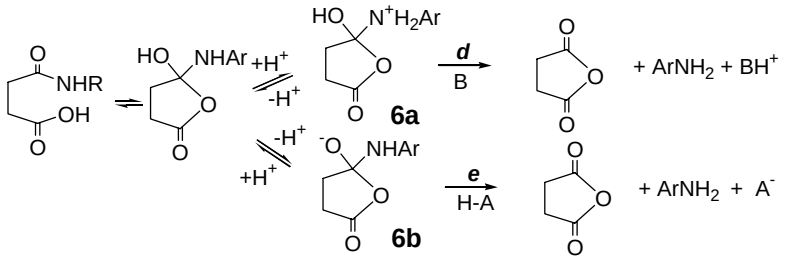
A lower extracellular pH (pH_e) is one of the few well-documented physiological differences between solid tumour and normal tissues. The mean pH_e in tumour tissues is 0.6-0.8 units lower than in normal tissues,[1,2] with an absolute value as low as 5.8,[3] considered to result from inefficient clearance of metabolic acids from chronically hypoxic regions. Because internal pH (pH_i) in cells is actively regulated, the tumor pH_e/pH_i differential can be considerable.[1,4] Prodrugs that could be selectively activated at the lower-than-normal pH_e occurring in tumour tissue thus have some theoretical advantages as drugs. Acetals have been studied in this regard, and do show substantial variation of cytotoxicity in the physiological pH range (6.2 to 7.4),[5,6] but are possibly too sensitive. Of more interest is the work with aconitic acid analogues as pH-sensitive spacers in the design of doxorubicin-armed antibodies, *e.g.* **5**.[7] These make use of the phenomenon that amides undergo accelerated hydrolysis with neighboring carboxylic acid group catalysis, through the pathway summarized in Scheme 1. The free acid group in **1a** allows the formation of the tetrahedral intermediate **2**, which breaks down slowly (stage ***b*** is rate-limiting) to form amine and anhydride. The latter hydrolyzes rapidly to dicarboxylic acid after amine release.[8]



Scheme 1

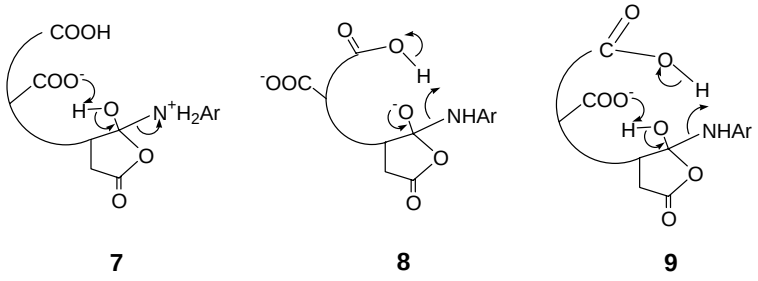
A potential problem with this design is that hydrolysis slows with increasing pH in the p*K*a region as the fraction of the non-reactive conjugate base form **1b** increases; with p*K*a values of carboxylic acids seldom being much above 4.5, monocarboxylic acids are normally nearly fully ionized around or below pH 5.5, and rates of amide hydrolysis become quite low at pH 6-7 as the fraction of the neighboring group in the catalytic free acid form drops away. However, for an amide with three carboxylic acid groups elsewhere in its structure, elevated second and third p*K*a values would ensure that in the monoanion or dianion there will be available at pH closer to 7 more undissociated COOH for the primary cyclization to form the tetrahedral intermediate (**2** in Scheme 1). Not only should this accelerate amide hydrolysis at neutral pH in its own right, but also there is potential for catalysis by the extra COOH and COO^- groups in breakdown of the tetrahedral intermediate **2**: for aryl amides, catalysis as shown in Scheme 2 is known for external (buffer) general bases (B) and acids (HA) through the pathways indicated.[8c]

Such catalysis could be provided intramolecularly by the basic COO^- group of an amide tricarboxylic acid in its monoanionic state (**7**; *cf* action of B on **6a** in step ***d*** in Scheme 2) or as the dianion (not shown) as well as by the acidic COOH group in the monoanion (**8**; *cf* action of HA on **6b** in step ***e*** in Scheme 2). Alternatively there could be combined intramolecular acid and base catalysis on the neutral tetrahedral intermediate (**9**). Subject to the acid and base groups being



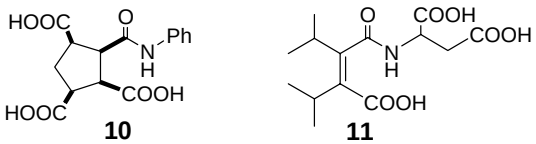
Scheme 2

suitably positioned to allow the required proton contacts to be made, these intramolecular catalytic effects may facilitate faster amide hydrolysis close to neutral pH.



7 **8** **9**

The above aspects of potential catalysis for an amide triacid encouraged the current exploratory study on the 3-*N*-phenyl-monamide **10** of *all-cis*-cyclopentane-1,2,3,4-tetracarboxylic acid as a potential scaffold for the preparation of pH-selective prodrugs. To our knowledge the only related amide with three proximate acid groups whose hydrolysis kinetics have been studied is the mono-amide of di-isopropylmaleic acid and aspartic acid (**11**), which hydrolyzes faster as both mono- and di-anion than in the neutral state.[8d]

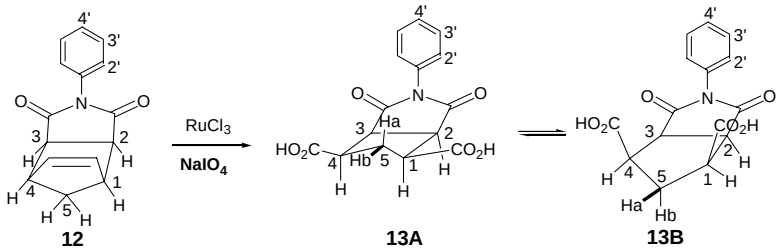


10 **11**

Results and Discussion

Synthesis and structure of the imides (3a*R*,4*R*,5*S*,6a*R*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,5-dicarboxylic acid (16) and (3a*R*,4*S*,6*R*,6a*S*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,6-dicarboxylic acid (13)

The symmetric imide dicarboxylic acid (**13**) was prepared (Scheme 3) from the Diels-Alder adduct (**12**) of *N*-phenylmaleimide and cyclopentadiene by $RuCl_3$/periodate oxidation across the 5,6-double bond following a method[9] used for the synthesis of *cis*-cyclopentane-1,3-dicarboxylic acid from norbornene, the method applied also to the preparation of the 2-methylphenyl analogue of **13**.[10] The only previous preparation of **13** involved oxidation of the same adduct using ozone.[11]



Scheme 3

The crystal structure of **13** as its monohydrate has two independent imide molecules in the asymmetric unit of the unit cell, each adopting an envelope conformation with the carboxylic acid groups in equatorial-like positions (*cf* **13A** not **13B**) as evident from one of the molecules depicted in Figure 1. The molecules differ slightly in the dihedral angle between the phenyl and imide rings (59.7 ° and 53.6 °), both values being close to the angle of 59.6 ° reported for *N*-phenylsuccinimide,[12] but otherwise are very similar structurally. The dihedral angles between C-H bonds along the C1-C5 axis as numbered in **13A** are referred to below in the context of coupling constants in the proton NMR spectrum of ionized **13** in D_2O. The two independent imide molecules are linked into pairs by the two lattice water molecules, acting an a H-bond acceptor from one carboxylic OH and as H-bond donors to each of an imide and a carboxyl C=O.

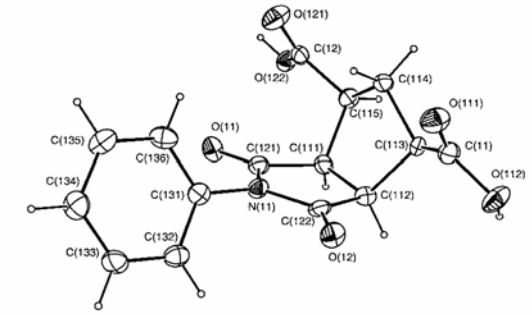
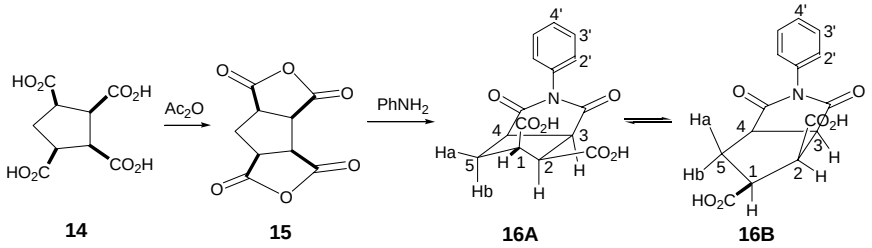


Figure 1. Perspective view of one of the two imide molecules in the unit cell of **13** showing crystallographic numbering

The unsymmetric imide dicarboxylic acid (**16**) was prepared by dehydrating (1*R*,2*R*,3*S*,4*S*)-cyclopentane-1,2,3,4-tetracarboxylic acid (**14**) to the 1,2;3,4-dianhydride (**15**) and reacting this with one mole equivalent of aniline in acetonitrile (Scheme 4).



Scheme 4

The crystal structure, determined on the methanol solvate, shows a similar conformational preference (**16A** not **16B**) to that for **13** (Figure 2). The dihedral angle between the phenyl and imide rings is larger in the **16** lattice (79.5 °) than in **13**. The methanol in the lattice acts as a H-bond donor to one of the imide carbonyl groups and as an acceptor from the 2-COOH group on an adjacent molecule. A further H-bond link is from the 1-COOH group in one molecule to the second imide C=O in the other.

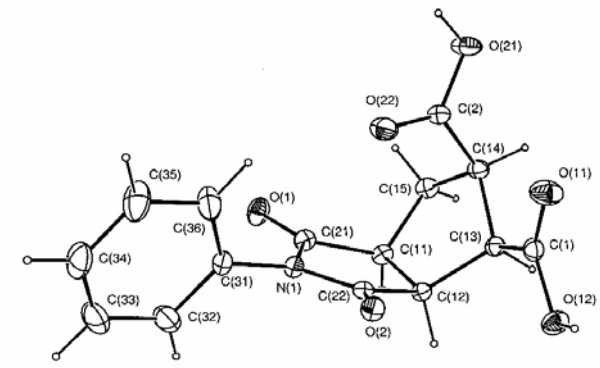


Figure 2. Perspective view of **16** showing crystallographic numbering.

NMR Spectra of 13 and 16

NMR spectral data for both imides are given in the experimental section for solution in DMSO-d_6 and D_2O. The spectra were also recorded in D_2O in the monoanion state (1 mole equivalent of NaOD added) and 1:1 mono- to di-anion state (1.5 mole equivalent of NaOD added) in order to be able to analyze spectra of the amide **10** in its ionized state as it reacts in D_2O to form these imides. The imide spectra were unchanged over time indicating that, even though ring opening to give the amide can occur in alkaline solution (see below), neither imide reacts in D_2O at this level of ionization and pD (6.5 for **13**; 7.1 for **16**). In passing, it is noted that these pD values

represent approximate second p*K*a values for **13** and **16** in D_2O and the higher value for **16** is expected because the two acid groups are in a 1,2-disposition in **16** rather than the 1,3-disposition in **13**; *cf*. *cis*-cyclopentane-1,2-dicarboxylic acid (second p*K*a 6.51) and *cis*-cyclopentane-1,3-dicarboxylic acid (second p*K*a 5.53).[13]

In the case of the symmetric imide **13**, previous ^{1}H NMR spectral assignments[11] were clearly deficient so a detailed analysis of the proton coupling patterns was carried out for the 1:1 mono- to di-anion solution in D_2O with a simulation to confirm assigned J values (see Experimental Section and Supplementary Information spectrum S1 for the simulation). Coupling constants between H1 and the two H5 protons (13 Hz and 5.4 Hz) are more consistent with conformation **13A** than **13B**; the latter would presumably be destabilized in the di-anionic state through closer proximity of the two carboxylate groups. In conformation **13A** strong coupling would be expected between H1 and H5a because of the large dihedral angle between the bonds C1-H1 and C5-H5a. In the crystal structure for the parent un-ionized diacid both molecules in the unit cell correspond to conformation **13A**, and the four dihedral angles range from 166.8 ° to 169.3 °. This fits well with the large coupling constant of 13 Hz, while the weaker (5.4 Hz) coupling is appropriate for H1 and H5b in **13A** because of the much smaller dihedral angle between the bond C1-H1 and C5-H5b (*cf* 44.8-46.9 ° in the crystal structure of the diacid molecules). In the other envelope conformation shown (conformation **13B**) with $C(5)H_2$ flipped to the opposite face of the cyclopentane ring, molecular models indicate that the dihedral angles between the bonds would be in the region of 80 ° (C1-H1 and C5-H5a) which is inconsistent with a coupling constant as high as 5.4 Hz by the Karplus relationship, and 40 ° (C1-H1 and C5-H5b) which is less consistent with the 13 Hz coupling than the larger dihedral angle for conformation **13A**.

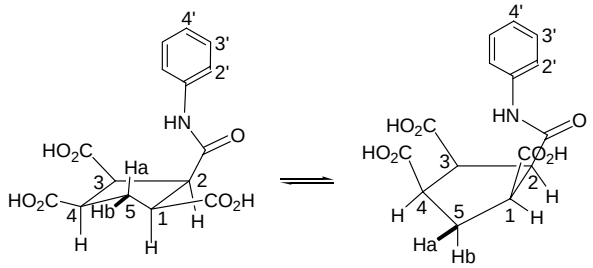
Kinetics of ring-opening imide hydrolysis of 13 and 16

An investigation into the kinetics of imide ring opening and the stability of the product amide triacid **10** in its trianionic state under alkaline hydrolysis was carried out to validate the following synthetic approach to **10**. Both imides **13** and **16** react readily as dianions in dilute hydroxide solution to give amide triacid with first order kinetics and a steady infinity reading by UV analysis (see Experimental Section for details and the second order rate coefficients). This indicates that the product amide triacid is not further hydrolyzed at a significant rate, possibly because the proximity of the three anionic carboxylate groups limits attack by hydroxide at the neighboring amide centre. Half-lives of the order of 1 minute for **16** and 2 minutes for **13** at 0.01 mol L^{-1} hydroxide showed that the reaction should be easily adapted to the synthetic scale. The symmetric imide **13** can give only **10**, but in the case of **16** it is not known if both isomeric amide triacids are formed; only **10** is separated on the synthetic scale (see below) so it is presumably the major product. Both this product preference and the rate difference between **16** and **13** can be explained by less electrostatic repulsion for hydroxide attack at the C4 imide carbonyl of the dianion of **16** whose nearest COO^- group is in a γ-position and more electrostatic repulsion for

hydroxide attack at the C3 imide carbonyl of the dianion of **16** and at the C3 (= C2) imide carbonyl of the dianion of **13**, each of which has a β-COO^- group.

Synthesis of the amide (1*S*,2*R*,3*R*,4*R*)-3-phenylaminocarbonylcyclopentane-1,2,4-tricarboxylic acid (10)

The imides **13** and **16** were treated with KOH solution which was dilute and in small excess (0.1 mol L^{-1}; *ca* 3.5 mole equivalents to allow for ionization in the triacid product) to reduce the chance of the amide product undergoing further hydrolysis. After reaction, acidification and immediate extraction with ethyl acetate to avoid acid-catalyzed hydrolysis gave the neutral amide tricarboxylic acid **10**. NMR spectra of the recrystallized product from both imides were identical and showed only one amide triacid to be present, establishing its identity as **10**, the only product available from the symmetric imide **13**. We have so far been unable to obtain crystals of **10** suitable for X-ray structure analysis, so no solid-state conformational preferences are available as a guide to whether **10A**, **10B** or other conformations might be dominant in solution.



NMR spectra and reaction monitoring of 10 in D_2O

(a) Un-ionized 10: NMR spectra of **10** as the free triacid reveal ready exchange of aryl ring protons with deuterium from solvent as shown by diminution over time of the aryl proton signals. The rate of loss of signal intensity for the *para* proton signal and the combined, overlapped *ortho* and *meta* signals is consistent with the two *ortho* and one *para* protons exchanging at similar rates while the *meta* proton is not exchanged. Exchange cannot be monitored to completion for confirmation of this specificity as the amide reacts simultaneously to form imides, and the exchange ceases in the imide products, presumably because the poorer donor nitrogen provides less *o,p*-activation to the electrophile D^+.

***(*b) Spectral changes and products from ionized 10 in unbuffered D_2O:** NaOD was added in varying quantities (1.6 mole: 40:60 mono- to di-anion; 2.15 mole: mostly dianion; 2.7 mole: 7:3 trianion to dianion) to D_2O solutions of **10** to investigate the spectral changes in its ionized states at higher pD. NMR spectra recorded on freshly prepared solutions showed differences in chemical shifts and coupling patterns for the CH signals of the cyclopentyl ring in **10** at different levels of ionization. Complete resolution of the four cyclopentyl CH protons H1-4 was obtained

on conversion largely to the trianion (2.7 mole NaOD added), with simplified coupling patterns relative to those at lower degrees of ionization (see Supplementary Information, spectrum S2(i)). When monitored over time, spectral changes showed hydrolysis to be largely swamped by imide formation, with a strong preference for the symmetric imide **13** in all three cases. There is no external buffering in the solutions obtained by adding NaOD, so that if the amide triacid **10** is initially in the dianion/trianion state, as it is for the solutions prepared with 2.15 and 2.70 moles of added NaOD, pD can be expected to increase when amide dianion gives imide dianion. This occurs because the latter is only a dicarboxylate species and is fully ionized so it can equilibrate only through proton abstraction, initially from un-ionized acid groups of unreacted **10** and, when that runs out, from water to produce hydroxide. The tetracarboxylic acid from hydrolysis would help with buffering but is only a minor product. The nett result is that pD will increase as the reaction occurs, influencing rate and product ratios. This effect was seen for the solution of **10** with 2.70 mole added NaOD (3:7 di- to tri-anion) which gave predominantly the symmetric imide **13**. After 6 hours about 75 % of **10** remained and after 48 hours about 50 % (see Supplementary Information, section S2). After another 24 hours little further reaction appeared to have occurred and the pD was measured and found to be 9.3. Clearly the reaction was stalling as pD increased and unreacted **10** was increasingly converted to its trianion. Also, on this time scale and while approaching such a high pD, the imides are possibly reactive enough, even with only low concentrations of OD^- present, to ring-open and thereby interconvert through the amide **10**, so the possibility of some pD-dependent equilibration amongst the three species exists. The apparent product ratio from the stalled reaction would therefore be irrelevant to kinetic control around pD 5-7. Similar, if not as extreme uncertainties relate to the other unbuffered solutions. In all cases, however, irrespective of the initial degree of ionization beyond the monoanion stage, NMR monitoring showed that the symmetric imide **13** was the clearly dominant product.

(c) Products and their rates of formation from 10 in D_2O with buffering: Reactions of **10** in D_2O solutions, when buffered by deuteroacetate and pyrophosphate buffers to avoid the loss of pD control referred to in *(b)*, revealed a significant difference in product ratio between pD 4.85 (deuteroacetate) and pD 6.45 (pyrophosphate) as determined by NMR analysis (see Supplementary Information sections S2-S5). Aliphatic protons H1-5 give distinctive signals in the two imide and the tetraacid products, but there is extensive overlap for mixtures. Fortunately, however, product ratio estimation is possible as follows (refer to spectra and integral values in Supplementary Information sections S4 and S5). First, the methylene proton signals (H5a and H5b) in **13** are clear of the corresponding, but overlapped, CH_2 signals from **16** and the tetraacid hydrolysis product (**14**) so the proportion of **13** can be assessed from integral measurements. Further, the tetraacid **14** has a characteristic *dd* signal for H2,3 which is sufficiently clear of all other CH signals to allow its separate integration. This two-proton integral value can be subtracted from that of the combined methylene CH_2 signal for **14** and **16** to obtain a two-proton integral for **16**. As shown in Supplementary Sections S4 and S5, these integral values provide **13**:**16**:**14** ratios of 35:40:25 at pD 4.85 (deuteroacetate buffer) and 65:25:10 at pD 6.45

(pyrophosphate buffer), with an estimated error in these figures of ± 5 given the limitations in accuracy of the integration analysis.

The proportion of **13** increases with pD at the expense of **16** and **14**, and to relate this to changes in rates of the three competing reactions of **10** required absolute rate measurement by UV spectroscopy. At the temperature (27 °C) and pD values very close to those in the NMR product study, the reaction of **10** gave k_1 7.3 x 10^{-5} s^{-1} at pD 5.02 (deuteroacetate buffer) and 3.0 x 10^{-5} s^{-1} at pD 6.53 (pyrophosphate buffer). These composite rate coefficients are the sums of the rate coefficients for the three parallel reactions of **10** and the individual rate coefficients can be obtained by splitting k_1 in proportion to product ratio for the appropriate pD. Because of inability to exactly match the pD values for kinetic work with those from the product study, the product data from pD 4.85 and 6.45 were used, making the assumption that the small pD differences from those of the kinetic study will not make much difference to product ratio. The rate coefficient for formation of **13** from **10** at pD 5.02 is therefore 35 % of 7.3 x 10^{-5} s^{-1}, *i.e.* approximately 2.5 x 10^{-5} s^{-1}. At pD 6.53 it is 65 % of 3.0 x 10^{-5} s^{-1} or approximately 2 x 10^{-5} s^{-1}, *i.e.* only a little reduced from that at pD 5 even though the nett rate for all three reactions of **10** is more than halved. Clearly the increased dominance of **13** between pD 5 and 6.5 results from its rate of formation being largely maintained with increasing ionization of **10** while rates for **14** and **16** decrease. By similar calculation (see Table 1) the rate coefficient for **16** drops about four-fold between pD 5 and 6.5 and for the hydrolysis product **14** the drop is calculated as about 7-fold although the experimental error will be larger here given lower accuracy in integration for the small quantity of **14** at the higher pD.

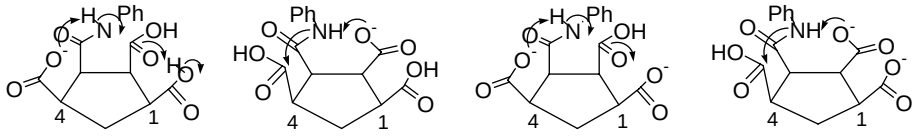
Table 1. Yield and rate coefficient calculation for the products from the reaction of **10**

pD		**13**	**16**	**14**
5.02 (deuteroacetate)	Yield / % (± 5)	35	40	25
	$10^5 k_1/s^{-1}$ (nett 7.3)	2.5	3	2
6.53 (pyrophosphate)	Yield / % (± 5)	65	25	10
	$10^5 k_1/s^{-1}$ (nett 3.0)	2	0.8	0.3

The decrease in the hydrolysis rate with increasing ionization in this amide triacid system is disappointing, in that even if imide formation were avoided by use of a tertiary amide, the rate of hydrolysis is likely to drop in the key pD 6-7 zone where it was hoped intramolecular catalysis in dianionic form would maintain a sufficient absolute rate for a pH-selective prodrug application (*ca* 10^{-4} – 5 x 10^{-5} s^{-1}, for a half-life under physiological conditions of *ca* 2-4 hours). The second question of whether a large rate differential between pH 6.5 and 7.5 might be obtained as a result of loss of intramolecular general acid-base catalysis with ionization to trianion, as outlined in the Introduction, is as yet unanswered for this system because the study was hindered by slow rates and competing imide formation. It would obviously be better pursued on a tertiary amide, but there would be a requirement for other skeletal modification to increase

overall rate through enhancing the initial neighboring group cyclization of the acid and amide groups to form the tetrahedral intermediate (**2**; Scheme 1). The norbornenyl[8c] and related bicyclic[14] systems are examples whose rigidity seems to ensure that these groups are held in effective proximity and they may hold most promise amongst succinate analogues for prodrug applications.

Perversely, one of the two imide-forming reactions competing with hydrolysis, that for **13**, does show just the effect we were seeking for hydrolysis in largely maintaining its rate with increased pD at least up to pD 6.5. Beyond pD 6.5 it is still the dominant reaction when **10** is mostly in its trianionic state even though the absolute rate is not known. In the monoanion state of **10**, because of the disposition of the groups involved in formation of **13**, C(1)-COOH could act as a general acid at C(2)-COOH to assist its being attacked by nucleophilic NH, and C(4)-COO^- could help as general base to remove the NH proton (**17**; note that here and in **18-20** the H atoms should strictly be D for reactions in D_2O). The first of these, the general acid effect, is not available for the formation of **16** because of the disposition of the groups (**18**). However, in the dianion state, while cyclization to a neutral acid group could be assisted by an ionized COO^- group to remove the NH proton from the other side of the amide group, this is available for the formation of both imides: for the symmetric imide **13** as in **19** and for the unsymmetric imide **16**, as in **20**. If anything, such assistance would be less favored for the formation of **13** through **19** because then the cyclization would occur with development of negative charge on C(2)-COOH immediately adjacent to the now-repelling C(1)-COO^-. The increasing dominance of **13** as a product as pD increases cannot therefore be explained by a disposition of catalytic groups in the dianion of **10** which is not accessible in the competing reaction to form **16**. Changes with increasing ionization in conformational preferences and in solvation factors may contribute.

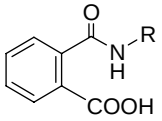


17 **18** **19** **20**

Summary

The above detailed study of the hydrolysis of **10** shows its preference for imide formation rather than amide hydrolysis in the weakly acid pH 5-7 region. At the time the work was commenced, such imide formation was not known to compete with hydrolysis for aryl amides with a neighbouring acid group, except by acid catalysis. For example, while the rate of acid-catalyzed (and reversible) imide formation exceeds the rate of hydrolysis in 1 molar acid for the 4-methoxy monoanilide of succinic acid,[8e] this is not observed above pH 2. To our knowledge, competing imide formation above pH 4 in water had been previously observed only for the *N*-methyl

monoamide of phthalic acid (**21**; R = Me; *N*-methylphthalamic acid),[8b] while not for phthalamic acid itself (**21**; R = H).[8a] A detailed study of this competition in an analogue with a second acid group on the aliphatic amine (**21**; R = CH_2COOH; *N*-(o-carboxybenzoyl)glycine) has been reported more recently.[15] Some detailed modelling has also been carried out on the competing imide-forming[16a] and hydrolysis[16b] mechanisms of the latter compound.



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Finally, the unexpected reactions of **10** in the pH 5-7 range described above show it is not suitable as a scaffold for the development of pH-sensitive prodrugs.

Experimental Section

General Procedures. Elemental analyses were performed at the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were measured on a Reichert Thermopan apparatus and are uncorrected. Infrared spectra were recorded as KBr disks on a Perkin-Elmer 1600 FTIR spectrometer. NMR spectra were run at 300.13 or 400.13 MHz (^{1}H), and 75.47 or 100.61 MHz (^{13}C) on Brüker AC-300, DRX-300 and DRX-400 spectrometers. Simulation of proton spectra was carried out with the program *gNMR* from Adept Scientific. Electrospray mass spectra were obtained on a VG Platform II spectrometer using samples prepared in 1:1 v/v MeCN/H_2O, a solvent that generates the 18 m.u. NH_4^+ ion, which appears in some diagnostic signals. A Uvikon 860 spectrophotometer with a thermostatted cell block was used for kinetic studies, monitoring absorbance changes of amide at 243 nm. Standardized KOH solutions used in the imide ring-opening kinetic studies were made up in nitrogen-flushed distilled water with ionic strength adjusted to 1.00 with added KCl. Deuteroacetate and pyrophosphate buffer solutions in D_2O for kinetic study of **10** were prepared similarly to those described below for the NMR study. First order rate coefficients k_1 were calculated from the gradients of log ($A_\infty - A_t$) *vs.* t and are estimated to be accurate to ±5 %. pH was measured with a Radiometer 26 meter fitted with a Schott Gerate H6180 combination electrode calibrated at appropriate temperatures using solutions prepared from commercial (BDH) buffer sachets dissolved in nitrogen-saturated distilled water. For solutions in D_2O, pD values were calculated from the pH meter reading by adding the standard[17] correction of +0.4 units.

For the NMR study of **13**, **16** and **10** in D_2O, solutions were normally prepared at about 0.05 mol L^{-1} in imide or amide, using D_2SO_4 (96-98 % w/w) from Merck (99.9 % D) and D_2O, CD_3COOD and NaOD (40 % w/w in D_2O) from Aldrich (99.8 % D). The NaOD solution was diluted to 0.39 mol L^{-1} with D_2O for use in preparation of the partially ionized imide and amide

solutions. The buffered 0.50 mol L^{-1} deuteroacetate solution of **10** was prepared with equal amounts of CD_3COOD and NaOD (pD 4.85 after addition of **10**) and the pyrophosphate buffered solution by adding 1.125 mol anhydrous $Na_4P_2O_7$ per mole of **10** providing buffering by $D_2P_2O_7^{2-}$ / $DP_2O_7^{3-}$ (final pD 6.45).

Apart from distillation of solvents, commercially sourced reagents were used without purification. *N*-Phenylmaleimide was prepared from aniline and maleic anhydride *via* maleanilic acid by small-scale adaptation of standard methods.[18] It had m.p 89 °C (lit.[18] 89.0-89.8 °C). A reference solution in D_2O of partly ionized (1*R*,2*R*,3*S*,4*S*)-cyclopentane-1,2,3,4-tetracarboxylic acid (**14**) was prepared by the addition of 2.5 mole equivalents of NaOD: ^{1}H NMR δ 3.47-3.35 (2H, dd, H2,3), 3.20-3.08 (2H, m, H1,4), 2.39-2.19 (2H, m, H5a, H5b); δ_C 180.3, 178.7 (both C=O), 52.4 (C1,4 or C2,3), 48.1 (C2,3 or C1,4), 32.5 (C5).

Synthesis and characterization of (3a*R*,4*R*,5*S*,6a*R*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,5-dicarboxylic acid (16). (1*R*,2*R*,3*S*,4*S*)-Cyclopentane-1,2,3,4-tetracarboxylic acid (**14**) (8.9 g, 0.037 mol) was dissolved with stirring in freshly distilled acetic anhydride (300 mL) heated to *ca* 120 °C. After 1 h the solution was cooled and most of the solvent was removed by rotary evaporation. The solid formed was filtered and washed with petroleum spirit (100 mL) to remove residual acetic anhydride. Drying over silica gel under vacuum gave **(1*R*,2*R*,3*S*,4*S*)-cyclopentanetetracarboxylic-1,2:3,4-dianhydride** (**15**; 7 g, 90 %) as cream colored crystals, m.p. 198 °C (lit.[19] 216 °C). IR (KBr) 1862, 1794 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 4.23-4.17 (2H, m, H2,3), 4.06-4.02 (2H, m, H1,4), 2.73-2.52 (2H, m, H5a, H5b). ^{13}C NMR (DMSO-d_6) δ 173.3 (C=O), 169.3 (C=O), 49.2 (C1 or C2), 48.9 (C1 or C2), 30.3 (C5). ESMS (MeCN, +30 V) *m/z* 228 ($M+NH_4^+$).

A solution of aniline (1.11 g, 0.012 mol) in dry acetonitrile (20 mL) was added dropwise to a stirred solution of the *cis*-dianhydride (2.50 g, 0.012 mol) in dry acetonitrile (100 mL) at 0 °C. The reaction mixture was stirred for 45 min. at 0 °C, then allowed to warm to room temperature overnight. The solvent was removed in vacuo to yield a yellow-orange gum, which was dissolved in a minimum volume of ethyl acetate at room temperature and crystallized by vapor diffusion of petroleum spirit (b.p. 63-65 °C). The resulting crystals were collected, washed with petroleum spirit (100 mL), and dried over silica gel under vacuum to yield anhydrous **(3a*R*,4*R*,5*S*,6a*R*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,5-dicarboxylic acid** (**16**; 3.49 g, 91 %), m.p. 192 °C; found: C, 59.11; H, 4.48; N, 4.80; calc. for $C_{15}H_{13}NO_6$: C, 59.40; H, 4.33; N, 4.62 %.). IR (KBr): 1778 (imide C=O), 1728 (carboxylic acid C=O), 1694 cm^{-1} (imide C=O). ^{1}H NMR (DMSO-d_6) δ 12.60 (br, COOH), 7.62-7.56 (2H, app. t, *J* 8.0 Hz, H3″), 7.52-7.46 (1H, app. t, *J* 8.0 Hz, H4′), 7.31-7.29 (2H, d, *J* 8.0 Hz, H2′), 3.85-3.80 (1H, app. t, *J* 9.2 Hz, H3), 3.67-3.60 (1H, app. q, H4), 3.56-3.52 (1H, app. t, H2), 3.38-3.32 (1H, app. q, H1), 2.46-2.34 (2H, m, H5a, H5b). ^{13}C NMR (DMSO-d_6) δ 178.1 and 176.0 (imide C=O), 173.0 and 172.9 (acid C=O), 132.8 (C1′), 128.8 (d, C2′), 128.3 (d, C4′), 127.1 (d, C3′), 48.9, 48.0, 47.6 (d, C2, C3, C4), 44.1 (d, C1), 29.5 (t, C5). ESMS (MeCN; +30 V) *m/z*: 304 ($M+H^+$), 321 ($M+NH_4^+$), 624 ($2M+NH_4^+$), 927 ($3M+NH_4^+$). Recrystallization from methanol by vapour

diffusion of diethyl ether gave crystals suitable for X-ray crystal structure determination as reported in the last part of this Experimental Section.

NMR Spectra of 16 in D_2O at different degrees of ionization. NMR spectra were recorded in D_2O in the neutral and some partially ionized states for comparison with spectra obtained when later monitoring the reactions of the amide triacid **9**.

^{1}H NMR (D_2O) δ 7.83-7.70 (3H, m, H3′, H4′), 7.51-7.41 (2H, H2′), 4.24-4.14 (1H, app. t, H3), 4.05-3.90 (2H, m, H2, H4), 3.72-3.60 (1H, app. q, H1), 2.85-2.58 (2H, m, H5a, H5b).

Mono-anion (D_2O + 1.0 mol added NaOD; pD 7.1): ^{1}H NMR δ 7.82-7.68 (3H, m, H3′,4′), 7.52-7.44 (2H, m, H2′), 4.07-3.98 (1H, app. t, H3), 3.90-3.80 (1H, m, H4), 3.80-3.73 (1H, app. t, H2), 3.56-3.45 (1H, app. q, H1), 2.78-2.65 (1H, m, H5a or H5b), 2.65-2.50 (1H, m, H5a or H5b); ^{13}C NMR δ 182.9 and 180.6 (imide C=O), 178.5 and 177.4 (acid C=O), 131.6 (s, C1′), 130.0 (d, C2′**)**, 130.0 (d, C4′), 127.3 (d, C3′), 51.2, 50.7, 48.3 (d, C2, C3, C4), 44.8 (d, C1), 30.4 (t, C5).

1:1 Mono- and di-anion (D_2O + 1.5 mol added NaOD; pD 7.1): ^{1}H NMR δ 7.82-7.65 (3H, m, H3′,4′), 7.52-7.45 (2H, m, H2′), 3.95-3.75 (2H, m, H3, H4), 3.68-3.58 (1H, app. t, H2), 3.42-3.30 (1H, m, H1), 2.78-2.64 (1H, m, H5a or H5b), 2.58-2.44 (1H, m, H5a or H5b); ^{13}C NMR δ 183.4 and 181.5 (imide C=O), 179.8 and 178.6 (acid C=O), 131.9 (s, C1′), 130.0 (d, C2′), 129.8 (d, C4′), 127.5 (d, C3′), 54.0, 52.5, 48.9 (d, C2, C3, C4), 45.2 (d, C1), 29.9 (t, C5). The spectrum was stable over time at this level of ionization and pD (7.1).

Synthesis and characterization of (3a*R*,4*S*,6*R*,6a*S*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,6-dicarboxylic acid (13). (i) Preparation of 12. To *N*-phenylmaleimide (19.7 g, 0.114 mol) dissolved in a minimum volume of tetrahydrofuran was added freshly distilled cyclopentadiene (9.4 mL, 0.114 mol) and the reaction mixture stirred at room temperature for 16 h. The resulting solid was collected and recrystallized from water/ethanol (1:1 v/v) to give *endo*-4-phenyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (**12**; 19.8 g, 70 %) as white rhomboid crystals, m.p. 143°C (lit.[11] 283 °C: product crystallized from dimethyl acetamide and its NMR data (DMSO-d_6) match those below). IR (KBr) 1769, 1700 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.44-7.35 (3H, m, H3′, H5′, H4′), 7.14-7.12 (2H, m, H2′, H6′), 6.26 (2H, s, H5, H6), 3.50-3.36 (4H, m, H1, H4, H2, H3), 1.79-1.59 (2H, m, H7a, H7b). ^{13}C NMR ($CDCl_3$) δ 176.8 (C=O), 134.7 (d, C5, C6), 132.0 (C1′), 129.1 (d, C2′, C3′, C4′) , 52.3 (t, C7), 45.8 and 45.6 (d, C2, C3 and C1, C4).

(ii) Oxidation of 12. $NaIO_4$ (28.8 g, 0.135 mol) and **12** (8 g, 0.036 mol) were added to a mixture of CCl_4 (67 mL), MeCN (67 mL) and water (100 mL) and the biphasic reaction mixture was stirred for 10 min. $RuCl_3.H_2O$ (160 mg, 7.1 mmol) was then added, and the mixture vigorously stirred for 2.5 h. Dichloromethane (100 mL) was added and stirring was continued for a further 10 min. to precipitate RuO_2. The aqueous phase was separated, filtered and retained. The organic phase was extracted with distilled water that was separated and filtered. The combined aqueous solution was refrigerated overnight to produce crude product collected as a grey mass (15 g). Recrystallization from boiling water/ethanol (2:1 v/v) produced **(3a*R*,4*S*,6*R*,6a*S*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,6-dicarboxylic acid** (**13**) as the monohydrate, white needles (4.15 g, 36 %), m.p. 219 °C (lit.[11] 253 °C for the anhydrous compound out of formic

acid); found C, 55.82; H, 4.54; N, 4.35; calc. for $C_{15}H_{13}NO_6.H_2O$: C, 56.08; H, 4.71; N, 4.36 %). IR (KBr) 1772 (imide carbonyl), 1740-1690 cm^{-1} (br, carboxylic acid and imide carbonyl). ^1H NMR (DMSO-d_6) δ 7.63-7.48 (3H, m, H3′, H4′), 7.36-7.24 (2H, m, H2′), 3.85-3.67 (2H, m, H2, H3), 3.31-3.15 (2H, m, H1, H4), 2.18-1.95 (2H, m, H5a, H5b). ^{13}C NMR (DMSO-d_6) δ 175.7 (C=O, imide), 172.6 (C=O, acid), 132.4 (C1′), 129.0, 128.5, 126.9 (C2′, C3′, C4′), 47.3, 45.6 (C2, C3 and C1, C4), 30.6 (C5). ESMS (1:1 MeCN/H_2O; +60 V) *m/z* 304 ($M+H^+$), 624 ($2M+NH_4^+$), 927 ($3M+NH_4^+$). The reported[11] ^1H and ^{13}C NMR spectra were consistent with these data but some signals were clearly misassigned. The crystal structure determination of **13** is reported at the end of the Experimental Section.

NMR Spectra in D_2O of 13 at different degrees of ionization. NMR spectra of **13** were recorded in D_2O in the neutral and partially ionized states similarly to the unsymmetric imide **16**. Because of inconsistency with the assignments made previously,[11] the proton spectrum of the compound as a 1:1 ratio of mono- and di-anion was analyzed in some detail to confirm assignments made here using coupling constants for alkyl CH and CH_2 protons in the cyclopentyl ring, and confirmed by simulation using the program *gNMR*.

^1H NMR (D_2O) δ 7.83-7.74 (2H, m, H1′, H4′), 7.50-7.42 (1H, m, H2′), 4.25-4.10 (2H, m, H2, H3), 3.68-3.54 (2H, m, H1, H4), 2.58-2.45 (1H, m, H5b), 2.40-2.23 (1H, app. q, H5a).

Mono-anion (D_2O + 1.0 mol added NaOD): ^1H NMR δ 7.82-7.70 (2H, m, H3′, H4′), 7.52-7.43 (1H, m, H2′), 4.01-3.90 (2H, m, H2, H3), 3.44-3.28 (2H, m, H1, H4), 2.40-2.28 (1H, m, H5b), 2.25-2.10 (1H, app. q, H5a); ^{13}C NMR δ 179.5 (C=O, imide), 178.0 (C=O, acid), 131.6 (C1′), 130.0, 129.9, 127.4 (C2′, C3′, C4′), 48.8, 48.0 (C2, C3 and C1, C4), 31.4 (C5).

Mono- and di-anion (D_2O + 1.5 mol added NaOD; pD 6.54): ^1H NMR δ 7.65-7.54 (2H, m, H3′, H4′), 7.35-7.30 (1H, m, H2′), 3.80-3.73 (2H, m, H2, H3), 3.20-3.10 (2H, m, H1, H4), 2.19-2.12 (1H, m, H5b), 2.03-1.92 (1H, app. q, H5a); ^{13}C NMR δ 179.8 (C=O, imide), 179.4 (C=O, acid), 131.6 (C1′), 130.0, 129.8, 127.5 (C2, C3′, C4′), 50.1, 47.9 (C2,3 and C1,4), 32.0 (C5). The spectrum was stable over time. Using the program *gNMR*, the following coupling constants for the alkyl protons H1-H5 in the cyclopentane ring were used to simulate a spectrum which gave a good fit with the observed splitting patterns at this level of ionization: 3.80-3.73 (2H, dd, $J_{2,1}$ = 9.5 Hz, $J_{2,3}$ = 7.0 Hz, H2, H3), 3.20-3.10 (2H, ddd, $J_{1,2}$ = 9.5 Hz, $J_{1,5a}$ = 13.0 Hz, $J_{1,5b}$ = 5.4 Hz, H1, H4), 2.19-2.12 (1H, dt, $J_{5b,5a}$= 12.5 Hz, $J_{5b,1}$ = $J_{5b,4}$ = 5.4 Hz, H5b), 2.03-1.92 (1H, dt, $J_{5a,5b}$ = 12.5 Hz, $J_{5a,1}$ = $J_{5a,4}$ = 13.0 Hz, H5a); see section S1 in Supplementary Information.

Kinetic study of ring-opening of 13 and 16 to form amide triacid. Ring-openings of both imides were followed by UV spectroscopy at 30.1 °C and ionic strength 1.0 (with KCl) in KOH solutions. For **13** values of 10^3 k_1 of 2.7, 5.6 and 11.2 s^{-1} at 0.0050, 0.0100, and 0.0200 mol L^{-1} KOH were obtained; and for **16** 10^3 k_1 of 5.9, 11.7 and 26.3 s^{-1} at the same concentrations of KOH. Plots of k_1 against hydroxide concentration gave as gradients the second order rate coefficients (k_2) 0.57 $Lmol^{-1}s^{-1}$ for (**13**) and 1.36 $Lmol^{-1}s^{-1}$ for (**16**).

Synthesis and characterization of (1*S*,2*R*,3*R*,4*R*)-3-(phenylaminocarbonyl)cyclopentane-1,2,4-tricarboxylic acid (10). Aqueous KOH (75 mL, 0.10 mol L^{-1}; 7.5 mmol) was added to **16** (0.66 g, 2.2 mmol) and the solution stirred for 100 min. The pH was rapidly reduced to below 2

by the addition of conc. HCl, followed by immediate extraction with ethyl acetate (3 × 100 mL). The combined extracts were washed with water (100 mL), dried over $MgSO_4$, and filtered. The solvent was evaporated and the white solid recrystallized from EtOH/*n*-heptane. The white crystals were collected and dried over silica gel under vacuum for one week. **(1*S*,2*R*,3*R*,4*R*)-3-(Phenylaminocarbonyl)cyclopentane-1,2,4-tricarboxylic acid (10**; 0.30 g; 43 %) had m.p.131-133 °C; found: C, 53.41; H, 5.14; N, 3.90; calc. for $C_{15}H_{15}NO_7.H_2O$: C, 53.12; H, 5.04; N, 4.13 % (calc. for $C_{15}H_{15}NO_7$ C, 56.01; H, 4.71; N, 4.36 %). IR (KBr): 1717 cm^{-1} (carboxylic acid carbonyl), 1622 cm^{-1} (secondary amide carbonyl). ^{1}H NMR (DMSO-d_6) δ 12.30 (br s, COOH), 10.12 (1H, s, NH), 7.61-7.59 (2H, d, *J* 8.0 Hz, H2'), 7.39-7.35 (2H, t, *J* 8.0 Hz, H3′), 7.14-7.12 (1H, t, *J* 8.0 Hz, H4′), 3.68-3.50 (2H, m, H2, H3), 3.17-3.08 (2H, m, H1, H4), 2.55 (m, obscured by DMSO signal, H5a or H5b), 2.24-2.18 (m, 1H, H5a or H5b). ^{13}C NMR (DMSO-d_6) δ 173.5, 173.1, 172.3, 170.6 (C=O), 139.0 (s, C1′), 128.4 (d, C3′), 123.2 (d, C4′), 119.6 (d, C2′), 48.9, 48.5, 45.9, 45.0 (C1, C2, C3, C4), 30.4 (C5). The NMR assignments for aryl H and C are made by analogy with the *N*-phenyl monoamide of succinic acid.[20] ESMS (MeCN, +60 V) *m/z* 322 ($M+H^+$), 643 ($2M+H^+$), 660 ($2M+NH_4^+$), 981 ($3M+NH_4^+$).

An equivalent hydrolysis starting from the symmetrical imide **13** but on a one-third scale gave a 30 % yield of the identical product as determined by NMR, white crystals, m.p 122 °C; the product was not dried under vacuum, elemental analysis was not performed, and its state of hydration is unknown. This confirms the crystallized product in both cases as **10**, even though the yield from the unsymmetrical imide **16** (*ca* 40 %) does not exclude the possibility that some of the alternative ring-opened isomer (1*S*,2S,3*R*,4*R*)-4-(phenylaminocarbonyl)cyclopentane-1,2,3-tricarboxylic acid is formed but does not crystallize with **10**.

NMR spectra in D_2O of 10 at different degrees of ionization. Spectra of **10** in D_2O without and with added NaOD provided ^{1}H NMR data for the neutral state as well as at different degrees of ionization**.** Subsequent spectral changes showed the formation of both imides **13** and **16** and hydrolysis products (see below), but also facile deuterium exchange occurred in the aromatic ring of the amide.

^{1}H NMR (D_2O) δ 7.40-7.25 (m, H2', H3'), 7.20-7.10 (m, H4'), 3.72-3.60 (2H, m, H2, H3), 3.35-3.21 (2H, m, H1, H4), 2.55- 2.25 (m, H5a, H5b). Signal intensity reduced over time for the aromatic H4' and overlapped H2' and H3' signals (see Results and Discussion section).

^{1}H NMR of a 40:60 mixture of monoanion and dianion (D_2O with 1.6 mol NaOD added) δ 3.95-3.70 (2H, m, H-2, H-3), 3.60-3.35 (2H, m, H1, H4), 2.70-2.55 (m, H5a, H5b).

^{1}H NMR of a 85:15 mixture of dianion and trianion (D_2O with 2.15 mol NaOD added) δ 3.95-3.75 (2H, m, H2, H3), 3.60-3.35 (2H, m, H1, H4), 2.73-2.52 (2H, m, H5a, H5b).

^{1}H NMR of a 30:70 mixture of dianion and trianion (D_2O with 2.7 mol NaOD added) 3.78-3.68 (1H, dd, J ~8Hz, 5Hz, H2 or H3), 3.65-3.57 (1H, app. t, J ~7.5 Hz, H2 or H3), 3.48-3.36 (1H, app. q, J ~7.5 Hz, H1 or H2), 3.30-3.18 (1H, app. dt, J ~8Hz, 7.5 Hz, H1 or H2), 2.57-2.47 (2H, app. t, J ~9 Hz, H5a, H5b); *cf.* initial spectrum S2(i) in Supplementary Information.

NMR monitoring of product formation from ionized 10 in D_2O without buffering. The reaction of **10** with 1.61 mol added NaOD was monitored to completion of reaction (*ca* 24 h at

27 °C). The imides **13** and **16** were formed along with hydrolysis products aniline and the tetraacid **14** as evident from the proton and carbon NMR spectra. In the latter, the ratio of peak heights at 31.7, 30.4 and 32.4 was about 4:2:1 giving a rough guide to the ratio of **13** to **16** to **14**, but this ratio cannot be related to a specific pD or degree of ionization of **10** as they will change during the course of the reaction. The same applies moreso for the solutions in which the triacid amide is already beyond the monoanion state (2.15 and 2.70 mol added NaOD) as discussed in the Results and Discussion section. For the latter case, see the sequential spectral changes in Supplementary Information section S2 which show the reaction stalling over time as pD increases and **10** becomes increasingly ionized.

NMR monitoring of product formation from ionized 10 in D_2O with buffering. Solutions of **10** buffered with pyrophosphate (pD 6.45) and deuteroacetate (pD 4.85), prepared as described in General Procedures, were monitored over time for product formation (see Supplementary Information section S3 for the pyrophosphate buffer case). At completion, ^{13}C-NMR spectra showed the correct number of alkyl C signals for products **13**, **14** and **16**, indicating the absence of any other byproduct: deuteroacetate-buffered product from **10** in D_2O (pD 4.85): δ 52.2 (**14**), 51.1 (**16**), 50.3 (**16**), 48.8 (**13**), 48.3 (**16**), 48.0 (**13**), 47.7 (**14**), 44.9 (**16**), 32.1 (**14**), 31.4 (**13**), 30.6 (**16**); pyrophosphate-buffered product from **10** in D_2O (pD 6.45): δ 53.4 (**14**), 53.0 (**16**), 52.1 (**16**), 50.0 (1**3**), 49.4 (**14**), 48.6 (**16**), 48.0 (**13**), 45.0 (**16**), 33.2 (**14**), 32.0 (**13**), 30.0 (**16**).

In the ^{1}H NMR spectra at completion, the CH_2 signals of **13** are separate from the overlapped CH_2 signals of **16** and **14**, and the characteristic H2,H3 *dd* CH signal of **14** is separate from other signals. Together, this allows for integration to estimate the product ratio (see the integration data and tables in sections S4 and S5 in the Supplementary Information, and Table 1 and discussion in the Results and Discussion section).

X-ray crystal structure determination of 13 and 16. Intensity data were collected on a Siemens SMART CCD diffractometer with monochromated Mo-Kα radiation (λ 0.7107 Å). Structures were solved by direct methods and routinely developed and refined (on F^2) using the SHELX-97 programs.[21]

(3a*R*,4*S*,6*R*,6a*S*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,6-dicarboxylic acid (13). White crystals of the monohydrate were obtained from water/ethanol. *Crystal data*: Empirical formula $C_{15}H_{15}O_7N$, M_r 321.28, orthorhombic, space group *Pna2₁*, *a* = 22.525(9), *b* = 7.103(3), *c* = 18.458(8) Å, *V* = 2953(2) Å^3, D_{calc} = 1.445 g cm^{-3}, *Z* = 8, μ(Mo Kα) = 0.116 mm^{-1}, size 0.40 x 0.21 x 0.11 mm, *F*(000) = 1344, *T* = 158 K. Total data 31212, unique data 6524 (R_{int} = 0.0732) in the range 1.81° ≤ θ ≤ 26.39°, 5961 with $I \geq 2\sigma(I)$, empirically corrected for Lorentz and polarization factors, but not for linear absorption. All non-H atoms anisotropic; H atoms in calculated positions, except for those of the water molecule, which were not apparent in final difference maps. R_1 ($I>2\sigma(I)$) = 0.0625, wR_2 (all data) 0.1650, GoF = 1.088, largest residual feature | 0.75 | e Å^{-3}. The structure is illustrated in Figure 1.

(3a*R*,4*R*,5*S*,6a*R*)-octahydro-2-phenyl-1,3-dioxocyclopenta[*c*]pyrrole-4,5-dicarboxylic acid (16). Translucent colourless crystals of the mono-methanol solvate were obtained from methanol/diethyl ether. *Crystal data*: $C_{16}H_{17}O_7N$, M_r 335.31, monoclinic, space group *P2₁/c*, *a*

= 10.7933(2), b = 11.7019(2), c = 12.9794(1) Å, β = 105.70(1)°, V = 1578.20(4) Å^3, D_{calc} = 1.411 g cm^{-3}, Z = 4, μ (Mo Kα) = 0.122 mm^{-1}, size 0.47 x 0.27 x 0.11 mm, F(000) = 704, T = 200 K. Total data 9420, unique data 3416 (R_{int} = 0.0179) in the range 1.96° ≤ θ ≤ 27.44°, 2877 with $I \geq 2\sigma(I)$, empirically corrected for absorption and other effects ($T_{max,min}$ 1.000, 0.810). All non-H atoms anisotropic: H atoms in calculated positions. R_I ($I>2\sigma(I)$) = 0.0413, wR_2 (all data) 0.1093, GoF = 1.040, largest residual feature | 0.23 | e Å^{-3}. The structure is illustrated in Figure 2. Full details of the structure determinations have been deposited with the Cambridge Crystallographic Data Centre as CCDC 276084 (**13**) and CCDC 276085 (**16**). Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or www: http//www.ccdc.cam.ac.uk).

Supplementary Information

S1. ^{1}H-NMR spectrum for **13** in D_2O with 1.5 mol equiv. NaOD added
S2. ^{1}H-NMR spectral changes for **10** in D_2O with 2.7 mol equiv. NaOD added
S3. ^{1}H-NMR spectral changes for **10** in D_2O buffered with pyrophosphate
S4. ^{1}H-NMR spectrum for **10** in D_2O buffered with pyrophosphate at completion with product ratio calculation from integration
S5. ^{1}H-NMR spectrum for **10** in D_2O buffered with deuteroacetate at completion with product ratio calculation by integration

Acknowledgements

We thank Dr Jan Wikaira, University of Canterbury, and Dr Cliff Rickard, University of Auckland, for collection of X-ray intensity data.

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