Synthesis of New Macrocyclic Amino-Phosphinic Acid Complexing Agents and Their *C*- and *P*-Functionalised Derivatives for Protein Linkage

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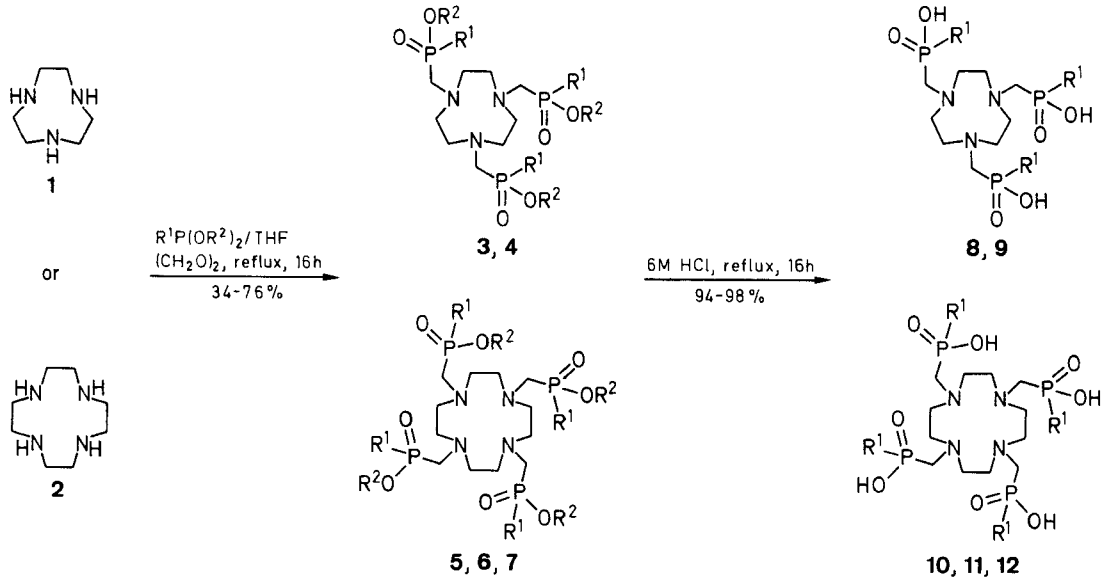
The synthesis of various macrocyclic complexing agents with alkyl- and arylphosphinic substituents is reported together with their *C*- and *N*-functionalised analogues as active esters suitable for antibody conjugation.

The development of macrocyclic metal complexes that are kinetically stable in vivo has accelerated a study of their use in diagnostic and therapeutic medicine.[1] Kinetically stable complexes of the imaging isotopes ^{111}In (γ, $t_{1/2}$ 2.81d) and ^{67}Ga (γ, $t_{1/2}$ 3.25d) have been reported using hexadentate ligands based on a triazacyclononane skeleton.[2] Octadentate ligands designed to bind the therapeutic radioisotope ^{90}Y (β, $t_{1/2}$ 64h) and the paramagnetic Gd^{3+} ion (for use in magnetic resonance imaging as a contrast agent[3]) have also been developed using a tetraazacyclododecane skeleton.[4,5] Until recently such ligands incorporated carboxymethyl groups to act as donor groups almost exclusively to satisfy the nuclear charge on the bound metal ion. An attractive alternative to the carboxylic acid donor group is a phosphinic acid. It is a stronger acid so that protonation not only of the free ligand but also of the phosphorus oxygen double bond in the metal complex is inhibited. The pentavalency of phosphorus means that an alkyl, aryl or other functionality may be introduced readily, permitting not only control over complex lipophilicity but also the introduction of a remote electrophilic site as is required if such ligands are to be used as bifunctional complexing agents in protein conjugation.[1,2,4] The synthesis of examples of hexa- and octadentate complexing agents incorporating alkyl and arylphosphinic acids is reported, together with their *C*- and *P*-functionalised analogous bearing remote active esters for conjugation to proteins.

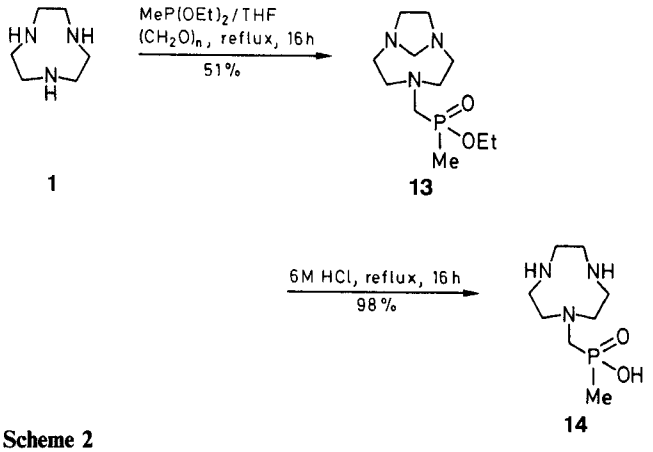
Condensation of 1,4,7-triazacyclononane **(1)** or 1,4,7,10-tetraazacyclododecane **(2)** with anhydrous paraformaldehyde in THF yielded an intermediate hydroxymethyl species which could be trapped with various dialkoxyphosphines to give the corresponding polymethylenephosphinate esters **3–7** (Scheme 1). Exclusion of moisture was essential to prevent formation of the hydroxymethylphosphinate esters, $HOCH_2PR(O)OR'$, and molecular sieves were used to scavenge any water. Attempts to promote the Arbusov reaction by deliberate addition of anhydrous tetrapentylammonium chloride or bromide failed to improve the yield of ester. In the case of triazacyclononane **1**, competitive formation of a bicyclic aminal monophosphinate ester, **13** (Scheme 2), occurred. The yield of this bicyclic compound was increased when the concentration of $MeP(OEt)_2$ was reduced. Related aminals have been reported previously with macrocyclic



Compound	R^1	R^2	Compound	R^1	R^2
3	Ph	Me	**8**	Ph	H
4	Me	Et	**9**	Me	H
5	Me	Et	**10**	Me	H
6	Ph	Me	**11**	Ph	H
7	Bu	Me	**12**	Bu	H

Scheme 1

polyamines.[6] Acidic hydrolysis of the aminal yielded the monosubstituted tetradentate ligand **14**, thereby permitting the synthesis of mixed donor ligands. Similarly, hydrolysis (6M HCl, reflux, 16h) of the esters **3–7** afforded the phosphinic acids **8–12** in essentially quantitative yield.

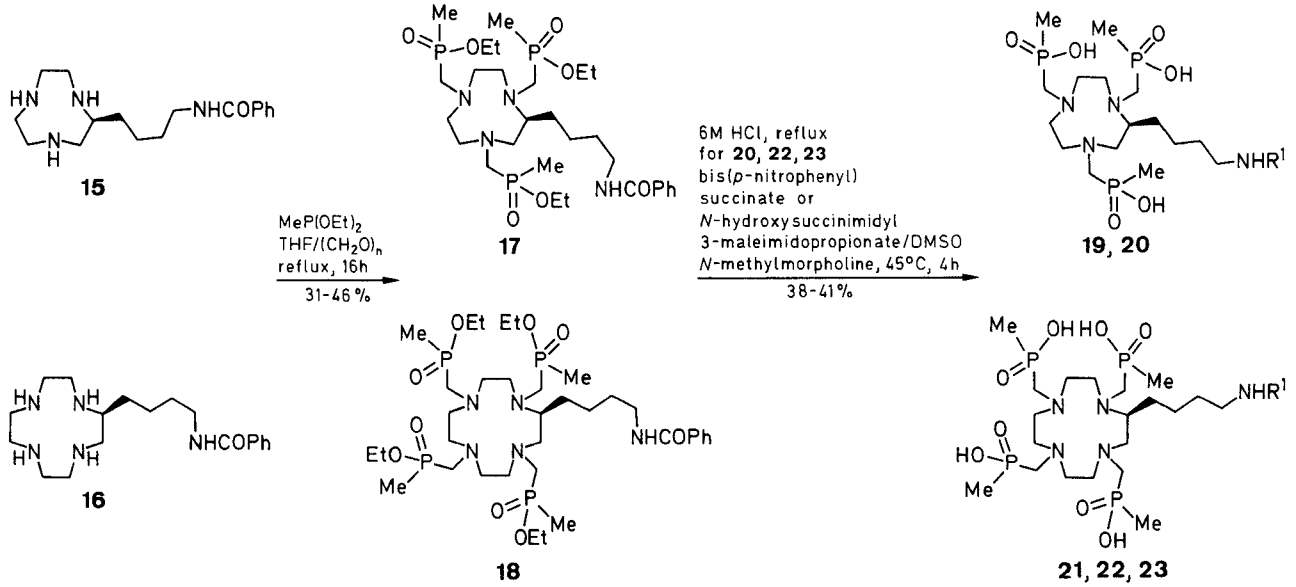


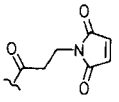
Scheme 2

This synthetic scheme was also used in the preparation of the *C*-aminoalkyl substituted analogues **19** and **21**. The 2-substituted benzamidobutyl polyamines **15** and **16**[5] were accordingly converted to the acids **17** and **18** in moderate yield. Again in the triazacyclononane series competitive formation of one of the two constitutionally isomeric bicyclic aminals occurred (10–20%). In order to conjugate these bifunctional complexing agents to a protein (or nucleotide), the pendant primary amine was reacted with a bifunctional linker molecule, such as a bis(*p*-nitrophenyl) succinate or *N*-hydroxysuccinimidyl-3-maleimidopropionate (Scheme 3). The resultant active esters **20** and **22** for example may be used directly to acylate lysine residues on an antibody (pH 8, phosphate buffer), with minimal protein aggregation.

The synthesis of the enantiopure precursor amines **15** and **16** from (2*S*)-lysine methyl ester involves a six-step procedure[4] and a shorter route to a functionalised ligand was sought using the parent polyamines as starting materials. With this in mind the mesylate **29** was prepared. It incorporates a protected amine group to allow subsequent protein conjugation. Radical addition of hypophosphorus acid across the olefinic bond of *N*-benzoylallylamine **(24)** (Scheme 4) followed by trapping of the intermediate alkylphosphonous acid with formaldehyde gave the hydroxymethylphosphinic acid **26**, isolated as its ammonium salt. Following ion exchange to the acid (Dowex 50 W, H^+), esterification with triethyl orthoformate yielded the ethyl ester **27**.

Competitive formation of mixed orthoformate **27** was observed, and this mixed ester could be separated by column chromatography on alumina and easily transesterified to the desired ester **28** in acidic ethanol. Mesylation of **28** in THF, rather than dichloromethane, afforded the mesylate **29** which was used to alkylate the [9]- and [12]-membered polyamines directly (Scheme 5), in moderate yield. Higher yields of **31** have been obtained by protection of three of the four nitrogens with a $Mo(CO)_3$ moiety prior to alkylation.[7] Transformation of the monosubstituted amines **30** and **31**, via the amino-acids **33** and

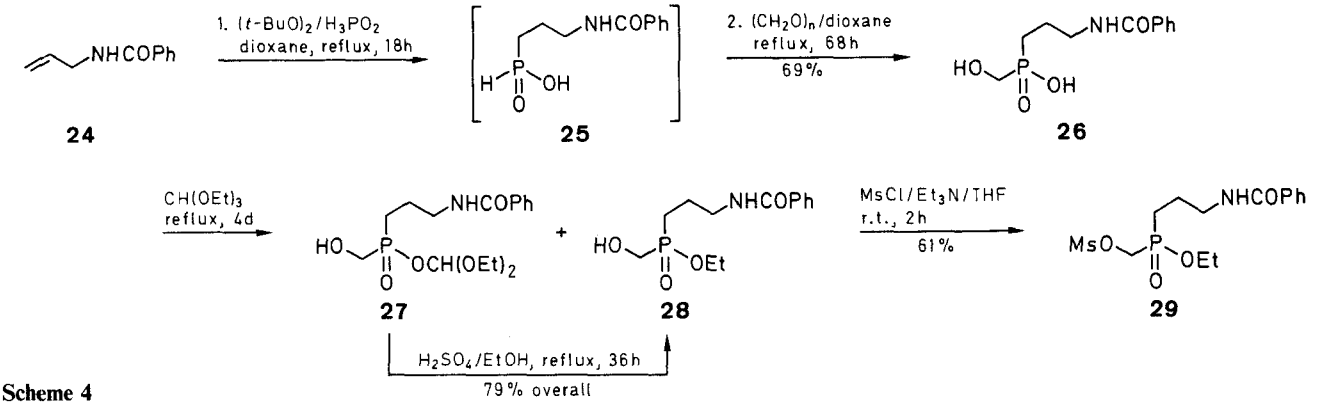


Compound	R^1	Compound	R^1
19	H		
20	$CO(CH_2)_2CO_2C_6H_4NO_2$-p	**23**	
21	H		
22	$CO(CH_2)_2CO_2C_6H_4NO_2$-p		

Scheme 3

35 to suitable acitve esters such as **36** could be achieved using the previously established methods.

Column chromatography was carried out using either 'gravity' silica (Merck Art 7734), 'flash' silica (Merck Art 9385), or neutral



Scheme 4

Table 1. Synthesis of Parent and *C*- and *P*-Functionalised Phosphinate Esters **4**–**7**, **17**, **18**, **32** and **34**

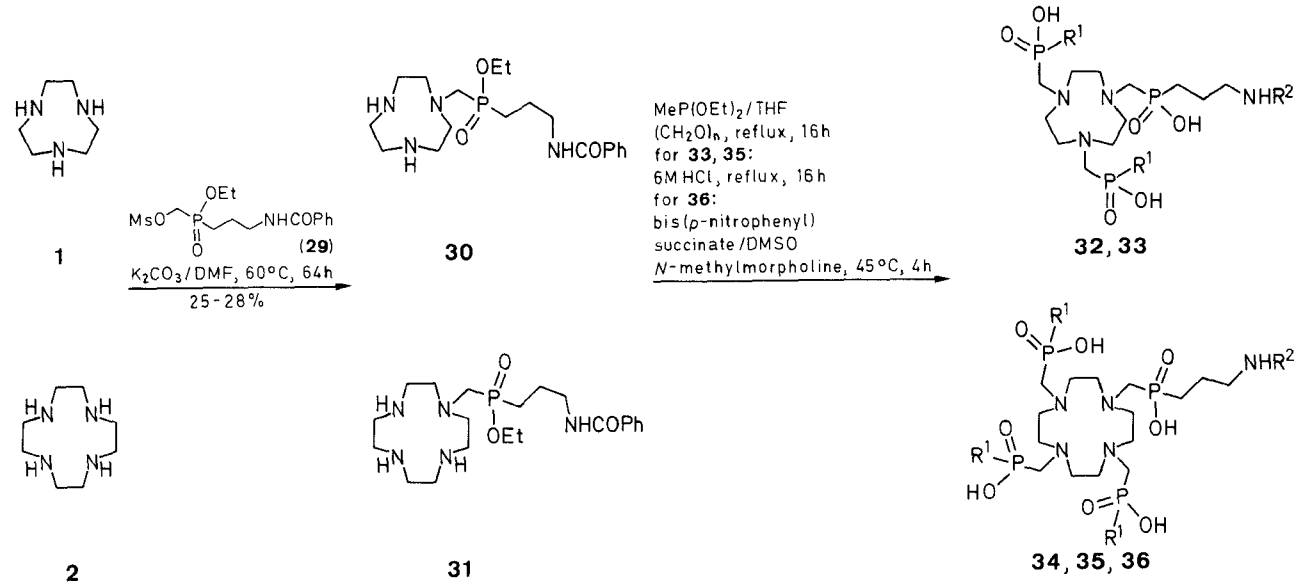
Starting Material	Product	Yield (%)	^{31}P NMR ($CDCl_3$) δ	^{1}H NMR ($CDCl_3$) δ, J (Hz)	^{13}C NMR ($CDCl_3$) δ, J (Hz)	Molecular Formula[a]
1	**4**	76	54.2, 54.13 (5 : 2)	1.32 (t, 9H), 1.54 (d, 9H, ^{2}J = 13.2, CH_3P), 3.0 (m, 12H, CH_2N), 4.08 (dq, 6H)	13.33 (d, J = 91, CH_3P), 16.61, 16.64, 16.66 (CH_3CH_2), 57.3, 57.4, 57.6, 57.7, 57.8, 58.4, 58.5 (CH_2N), 60.1, 60.2 (CH_2O)	$C_{18}H_{42}N_3O_6P_3$ (489.2)
2	**5**	50	51.9, 51.8, 51.6 (diastereomers)	1.31 (t, 12H), 1.57 (d, 12H, ^{2}J = 13.7), 2.64–3.07 (m, 24H, CH_2N), 4.07 (dq, 8H, CH_2O)	13.44 (^{1}J = 91, CH_3P), 16.42 (d, ^{3}J = 5), 54.18, 54.30 (d, ^{1}J = 110), 59.82 (d, ^{2}J = 6)	$C_{24}H_{56}N_4O_8P_4$ (652.3)
2	**6**	37	41.5	2.1–2.9 (br m, 24H, CH_2N), 3.56 (d+d+d+d, 12H, CH_3O isomers), 7.45 (m, 12H), 7.78 (m, 8H, *o*-Ar)		$C_{44}H_{64}N_4O_8P_4$ (900.4)
2	**7**	46	53.8	0.97 (t, 12H), 1.24 (t, 12H), 1.35 (dt, 8H, CH_2C), 1.50 (m, 8H, CH_2C), 1.7 (br m, 8H, CH_2P), 2.6–2.95 (br, 24H, CH_2N), 4.01 (dq, 8H, CH_2O)	13.49 (CH_3CH_2C), 16.70 (d, ^{3}J = 4.4), 23.75, 23.88, 24.04 (CH_2C), 27.35 (d, ^{1}J = 87, CH_2P), 53.28 (d, ^{1}J = 104, CH_2N), 53.95, 54.02, 54.10 (CH_2N), 60.01 (d, ^{2}J = 4, CH_2O)	$C_{36}H_{80}N_4O_8P_4$ (820.5)
15	**17**	30	52.3, 51.4, 50.3, 49.9 (2 diastereomers)	1.20 (t+t, 9H), 1.30 (m, 9H), 1.55–1.42 (m, 4H, CH_2CH), 1.63 (m, 2H, CH_2CH_2NHCO), 3.50–2.60 (m, 19H, CHN + CH_2N), 4.02 (m, 6H), 7.45–7.39 (m, 3H, *o*- and *p*-Ar), 7.85 (br t, 1H NHCO), 7.91 (dd, 2H, *o*-Ar)	12.95 + 12.80 (d+d, J = 90, 91), 13.22 (d, ^{1}J = 89 major isomer), 16.40, 16.36 (CH_3), 24.0, 23.9 (CH_2C), 28.6 (CH_2C), 39.4, 39.35 (CH_2NHCO), 52.7, 53.0, 53.1, 57.0, 57.8, 57.9, 58.15, 58.2, 59.0, 59.9 (CH_2N), 63.5 (CH_2O), 127.1, 121.9, 130.7, 134.75, 167.4	$C_{29}H_{55}N_4O_7P_3$ (664.3)
16	**18**	46	52.3, 52.2, 52.1, 52.0, 51.6, 50.5, 50.4	1.18–1.23 (m, 12H, CH_3), 1.25–1.50 (m, 18H, CH_3P + CH_2C), 2.23–3.82 (m, 25H), 3.93–4.05 (dq+dq+dq, 8H, CH_2O), 7.34 (m, 3H), 7.66 (br t, 1H, NHCO), 8.07 (dd, 2H, *o*-Ar)	16.1 + 15.9 (d+d, J = 91, CH_3P), 19.2 (CH_3), 26.4, 29.5, 29.6 (CH_2C), 40.9 (CH_2NHCO), 49.7, 51.0, 51.5, 51.7, 54.7, 55.0, 55.9, 56.3, 56.8, 58.0, 58.1, 58.2, 59.2 (CH_2N), 60.5 (CH_2O), 124.7, 125.5, 128.3 (Ar, C-H), 132.5 (s), 164.8 (NHCO)	$C_{35}H_{69}N_5O_9P_4$ (827.4)
30	**32**	36	52.4, 51.9, 51.7, 50.4, 50.0	1.30 (t+t, 9H), 1.51 (d, 6H, CH_3P), 1.98 (br m, 4H, $CH_2CH_2PO_2Et$), 2.85–3.05 (m, 20H, CH_2N), 4.07 (dq+dq+dq, 6H, CH_2O), 7.45 (m, 3H), 7.91 (dd, 2H), 8.10 (br t, 1H, NHCO)		$C_{27}H_{51}N_4O_7P_3$ (636.3)
31	**34**	40	52.3, 52.1, 52.0, 51.8, 50.6, 505.	1.30 (t, 12H, J = 7.2), 1.49 (d+d+d, 9H, PCH_3), 1.80–3.70 (br m, 30H, CH_2N + CH_2P + CH_2C), 4.05 (dq, 8H, CH_2O), 7.39 (m, 3H), 7.92 (dd, 2H, *o*-Ar), 8.35 (br t, 1H, NHCO)		$C_{33}H_{65}N_5O_9P_4$ (799.4)

[a] Accurate masses were obtained (DCI, MeOH) with ±0.0007 amu, except for **34** and **18** ±0.0009 amu.

Table 2. Synthesis of Phosphinic Acids **9–12**, **19**, **21**, **33** and **39**

Starting Ester	Product	Yield (%)	^{31}P NMR (D_2O, pD as stated) δ	^1H NMR (D_2O) δ, J (Hz)	^{13}C NMR (D_2O) δ, J (Hz)	Molecular[a,c] Formula
4	**9**	89	pD 0.0: 50.0	1.42 (d, 9H, ^2J = 14), 3.32 (br d + s, 18H, CH_2N)	15.43 (d, ^1J = 93), 51.60, 55.06 (d, ^1J = 92)	$C_{12}H_{30}N_3O_6P_3$ (405.1)
5	**10**	93	pD 14: 39.2	pD 6.5: 1.41 (d, 12H, J = 14.1), 3.37 (br, 24H, CH_2CN)	pD 1: 14.86 (d, ^1J = 94), 50.70 (CH_2N), 51.64 (d, ^1J = 118)	$C_{16}H_{40}N_4O_8P_4$ (540.2)
6	**11**	88	pD 14: 28.0	pD 10: 2.06 (m, 16H, CH_2N), 2.26 (br d, 8H, CH_2N), 7.25 (m, 12H), 7.46 (m, 8H, *o*-Ar)	49.60, 56.01 (d, ^1J = 98), 128.3 (br), 130.9 (br), 137.07 (d, ^1J = 118)	$C_{36}H_{48}N_4O_8P_4$ (788.2)
7	**12**	95	46.83[d]	0.97 (t, 12H), 1.4–1.7 (m, 16H, CH_2C), 1.95 (dt, CH_2CH_2P), 3.4–3.8 (br m, 24H, CH_2N)[d]	13.95 (CH_3), 24.11, 24.16, 24.79, 24.95 (CH_2C), 29.61 (d, ^1J = 96, CH_2P), 52.4 (br d), 52.9 (br, CH_2N)[d]	$C_{28}H_{64}N_4O_8P_4$ (708.4)
17	**19**	[b]	pD 13.5: 40.4, 40.2 (13.5)	1.46 (d + d, 9H, ^2J = 14), 1.5–1.8 (br m, 6H, CH_2C), 2.97 (t, 2H, CH_2NH_3), 3.0–3.6 (m, 17H, CH_2N + CHN)	–	$C_{16}H_{39}N_4O_6P_3$ (476.2)
18	**21**	[b]	pD 13: 39.4, 39.3, 39.2	1.37 (d + d + d, 12H, J = 14), 1.36–1.60 (br m, 4H, CH_2C), 1.55–1.75 (br m, 2H), 2.88 (t, 2H, CH_2NH_3), 2.6–3.7 (m, 23H, CHN + CH_2N)	–	$C_{20}H_{49}N_5O_8P_4$ (611.25)
32	**33**	[b]	pD 13.4: 40.6, 40.3, 39.9	1.43 (d, 6H, J = 13.5, CH_3P), 1.6–1.8 (m, 4H, CH_2CH_2P), 2.99 (t, 2H, CH_2NH_3), 3.4–3.7 (m, 18H, CH_2N + CH_2P)	–	$C_{14}H_{35}N_4O_6P_3$ (448.2)
34	**35**	[b]	pD 13: 39.3, 39.0, 38.9	1.40 (d, 9H, J = 14, CH_3P), 1.73 (m, 2H, $CH_2CH_2NH_2$), 1.86 (m, 2H, CH_2CH_2P), 2.98 (t, 2H, CH_2NH_3), 3.25–3.60 (br m, 24H, CH_2N + CH_2P)	–	$C_{18}H_{45}N_5O_8P_4$ (583.2)

[a] Molecular ions were observed using negative ion FAB mass spectrometry (3-nitrobenzyl alcohol).
[b] Conversion was quantitative as deduced by 400 MHz ^1H NMR (Varian VXR-400).
[c] Isolated yield of the hydrochloride salts, analyses were C ±0.35, H ±0.27, N ±0.31.
[d] Solvent: MeOD.



Compound	R^1	R^2	Compound	R^1	R^2
32	Et	COPh	**35**	H	H
33	H	H	**36**	H	$CO(CH_2)_2CO_2C_6H_4NO_2$
34	Et	COPh			

Scheme 5

alumina (Merck Art 1077) which had previously been treated with EtOAc. Analytical and semi-preparative HPLC was performed with a Varian Vista 5500/Polychrome 9060 instrument fitted with either cation exchange ('Synchropak' CM 300), anion exchange ('Synchropak' AX 100) or reverse phase columns ('Spherisorb' 5 ODS2). Flow rates of 1.4 mL min^{-1} and 4.0 mL min^{-1} were used for analytical and semi-preparative columns respectively. Column and gradient elution conditions were as follows: cation exchange, t = 0 min, 80% H_2O, 0% aq NH_4OAc (1.0 M, pH 5.6), 20% MeCN; t = 5 min, 60% H_2O, 20% aq NH_4OAc, 20% MeCN; t = 10 min, 0% H_2O, 80% aq NH_4OAc, 20% MeCN. For anion exchange: t = 0 min, 70% H_2O, 10% aq NH_4OAc, 20% MeCN; t = 20 min, 0% H_2O, 80% aq NH_4OAc, 20% MeCN. For reverse phase: t = 0 min, 95% H_2O, 0% aq NH_4OAc, 5% MeCN, t = 20 min, 5% H_2O (0.1% trifluoroacetic acid) 0% NH_4OAc, 95% MeCN (0.1% trifluoroacetic acid). Solvents used were dried from an appropriate drying agent, and water was purified by the Milli Q system. IR spectra were recorded with a Perkin Elmer 577 spectrometer, ^1H, ^{13}C and ^{31}P NMR spectra were obtained with a Bruker AC 250 operating at 250.13, 62.90 and 101.1 MHz, respectively. Mass spectra were recorded with a VG 7070E spectrometer operating in CI, DCI, or FAB modes with DCI samples presented as dilute CH_2Cl_2 or MeOH solutions and ammonia as the impingent gas. *m*-Nitrobenzyl alcohol or glycerol was the matrix for FAB analyses.

Trimethyl 1,4,7-Triazacyclononane-1,4,7-triyltris[methylene(phenylphosphinate)] (3); Typical Procedure:

To a solution of 1,4,7-triazacyclononane **(1)** (0.3 g, 2.4 mmol) in THF (40 mL) was added phenyldimethoxyphosphine (1.8 g, 12 mmol) and paraformaldehyde (0.45 g) and the mixture was heated to reflux (16 h) in a Soxhlet apparatus equipped with 3 Å molecular sieves. After removal of solvent under reduced pressure the residue was purified using column chromatography on neutral alumina (0 to 5% MeOH in CH_2Cl_2) to yield **3** as a colourless oil which was dried at 25 °C, 10 mbar; yield: 1.14 g (75%); R_f 0.58 (alumina, 10% MeOH in CH_2Cl_2).

MS (DCI): *m/z* = 635 (M + 2), 634 (M + 1).

^1H NMR ($CDCl_3$): δ = 2.70 (m, 12 H, CH_2N ring), 2.98 (d, 6 H, ^2J = 7 Hz, CH_2P), 3.65 (d, 9 H, ^3J = 11 Hz, OCH_3).

^{13}C NMR ($CDCl_3$): δ = 50.55 (d, ^2J = 7 Hz, OCH_3), 56.3 (m, ring CH_2N), 56.6 (d, J = 119 Hz, CH_2P), 129.6 (d, ^1J = 118 Hz), 128.05 (d, ^2J = 12 Hz, *o*-C), 131.65 (d, ^3J = 10 Hz, *m*-C), 132.2 (*p*-C).

^{31}P NMR ($CDCl_3$): 40.74 (s).

Data for other esters prepared in a similar manner are given in Table 1.

1,4,7-Triazacyclononane-1,4,7-triyltris[methylene(phenylphosphinic Acid)] (8); Typical Procedure:

The trimethyl ester **3** (0.4 g, 0.63 mmol) was dissolved in hydrochloric acid (6 M, 15 mL) and the solution was heated to reflux for 16 h. Concentration of the solution to small volume and adjustment of the pH (KOH solution) to 2, led to formation of **8** as a colourless solid which was filtered and dried (25 °C, 10 mbar); yield: 0.34 g (77%).

$C_{27}H_{33}N_3P_3O_6 \cdot 2HCl \cdot 2H_2O$ (696.1)	calc.	C 46.29	H 5.57	N 6.00	Cl 10.19
	found	46.01	5.75	6.07	9.09

MS (FAB): *m/z*: 588 (M), 587 (M-1).

^1H NMR (D_2O, pD 0.5): δ = 3.10 (br, 12 H, CH_2N ring), 3.25 (d, 6 H, ^2J = 7 Hz, CH_2P), 7.5 (br, 9 H, *m*- + *p*-CH), 7.60 (m, 6 H, *o*-CH).

^{13}C NMR (D_2O, pD 0.5): δ = 51.6 (CH_2N), 55.4 (d, ^1J = 98 Hz, CH_2P), 129.9 (d, ^1J = 100 Hz, *ipso*-C), 130 (br, *m*-Ar), 132 (br, *o*-Ar), 133.8 (br, *p*-Ar).

^{31}P NMR (D_2O, pD 0.5): δ = 27.2.

Data for other acids prepared in a similar manner are given in Table 2.

4-Ethoxy(methyl)phosphorylmethyl-1,4,7-triazabicyclo[5.2.1]nonane (13):

This compound was formed during the synthesis of **4**, and was prepared in higher yield using an analogous procedure from triazacyclononane and diethoxy(methyl)phosphine in equimolar amounts; yield 51%.

MS (DCI): *m/z* = 262 (M + 1); calc. for $C_{11}H_{24}N_3O_2P$: 261.16061, found: 261.16042.

$C_{11}H_{24}N_3O_2P$ (261.2)	calc.	C 50.54	H 9.19	N 16.08	
	found	50.31	9.01	15.84	

^1H NMR ($CDCl_3$): δ = 1.33 (t, 3 H, OCH_2CH_3), 1.53 (d, 3 H, CH_3P), 2.80 (m, 4 H, CH_2N), 3.05 (m, 8 H, CH_2N), 3.24 (ddd, 2 H, CH_2P), 4.09 (dq, 2 H, CH_2O), 4.16 (dd, 2 H, J = 10.4 Hz, NCH_2N).

^{13}C NMR ($CDCl_3$): δ = 12.69 (d, ^1J = 89, CH_3P), 16.53 (d, ^3J = 5 Hz, CH_3), 49.10, 49.05 (CH_2N, 5-ring), 54.02, 54.00 (CH_2N, 8-ring), 55.71 (d, J = 5.3 Hz, CH_2NCH_2), 55.94 (d, J = 6.1 Hz, NCH_2CH_2P), 55.97 (d, ^1J = 114 Hz, CH_2P), 60.10 (d, ^2J = 6.8 Hz, CH_2O), 76.16 (NCH_2N).

1,4,7-Triazacyclononane-1-yl-methylene(methylphosphinic Acid) (14):

This was prepared from **13** as described for the preparation of **8**, and was isolated as the hydrochloride salt as a colourless glass; yield 98%.

MS (FAB): *m/z* = 222 (M^+ + 1).

$C_8H_{20}N_3O_2P \cdot 2HCl \cdot 2H_2O$ (329.1)	calc.	C 29.06	H 6.06	N 12.72	Cl 21.50
	found	28.81	6.31	12.61	21.21

^1H NMR (D_2O, pD 1): δ = 1.33 (d, ^2J = 13.6 Hz, CH_3P), 2.95 (t, 4 H, ^2J = 6 Hz, CH_2N), 2.99 (d, 2 H, ^2J = 4 Hz, CH_2P), 3.13 (t, 4 H, CH_2N), 3.45 (s, 4 H, NCH_2CH_2N)

^{13}C NMR (D_2O, pD 1): δ = 14.58 (^1J = 85.5 Hz, CH_3P), 42.91 (CH_2N), 44.54 (CH_2N), 50.48 (^3J = 4.9 Hz, CH_2NCH_2P), 54.04 (^1J = 101.5 Hz, CH_2P)

3-Benzamidopropyl(hydroxymethyl)phosphinic acid (26):

To a solution of *N*-benzoylallylamine **24** (7.47 g, 46.4 mmol) in dioxane (100 mL) was added hypophosphorous acid (8.66 g, 50% aq. sol) and *tert*-butyl peroxide (0.3 g) and the mixture was heated to reflux for 18 h. After removal of solvent under reduced pressure, ^1H NMR analysis of the crude residue revealed the disappearance of the olefinic resonances. The residue was redissolved in dioxane (50 mL) and excess paraformaldehyde (20 g) was added and the mixture heated to reflux for 68 h. After removal of solvent, the residue was purified by chromatography on silica gel (eluant 70% CH_2Cl_2, 28–25% MeOH, 2 → 5% aq NH_4OH) to yield the ammonium salt of the acid **26** as a hygroscopic colourless glass, yield: 9.12 g (69%).

MS (FAB): *m/z* = 257 (M), 256 (M-1).

^{13}C NMR (D_2O): δ = 22.03 (CH_2CH_2P), 25.12 (d, ^1J = 81 Hz, CH_2P), 41.01 (CH_2NHCO), 59.73 (d, ^1J = 99 Hz, PCH_2OH), 127.22, 128.98, 132.28 (Ar), 134.0 (s), 170.04 (CONH)

^{31}P NMR (D_2O): δ = + 41.1 (s).

Ethyl 3-Benzamidopropyl(hydroxymethyl)phosphinate (27):

To a solution of the ammonium salt of **26** (5 g) in water (25 mL) was added a strong acid ion-exchange resin (Dowex 50W, H^+ form, 30 g) and after filtration and evaporation, the residue was treated with triethyl orthoformate (25 mL) and the mixture heated to reflux for 96 h. After evaporation, the residue was purified by silica gel chromatography (5 to 10% MeOH/CH_2Cl_2) to yield a mixture of the desired ester **27** and its mixed orthoformate ester **27** [^{31}P NMR ($CDCl_3$): δ = 51.68; MS(CI): *m/z* = 387 (M)]. Transesterification of this mixture was effected by boiling in EtOH (100 mL) in the presence of conc. H_2SO_4 (1 mL) for 36 h. Evaporation and purification by silica gel chromatography yielded **27** as a pale yellow oil; overall yield: 4 g (79%).

$C_{13}H_{20}NO_4P$	calc.	C 54.72	H 7.02	N 4.91	P 10.87
(285.1)	found	54.45	7.30	5.01	10.64

MS (DCI): m/z = 286 (M + 1).

^{1}H NMR ($CDCl_3$): 1.05 (t, 3H, CH_3), 1.75 (m, 4H, CH_2CH_2P), 3.31 (dt, 2H, CH_2NHCO), 3.70 (br d, 2H, CH_2OH), 3.81 (dq, 2H, CH_2O), 4.05 (br s, 1H, OH), 6.85 (br t, 1H, NHCO), 7.25 (m, 3H), 7.71 (dd, 2H, *o*-Ar).

^{13}C NMR ($CDCl_3/CD_3CO_2D$; 1:1) δ = 15.37 (CH_3), 20.33 (CH_2), 21.64 (d, ^1J = 90 Hz), 39.62 (CH_2NHCO), 56.16 (d, ^1J = 109 Hz, CH_2OH), 62.1 (CH_2O), 126.6, 127.8, 131.1 (d); 132.98 (s), 168.56 (CONH).

^{31}P NMR ($CDCl_3$): δ = + 53.7.

Ethyl 3-Benzamidopropyl(mesyloxymethyl)phosphinate, (29):
To a suspension of the alcohol **27** (0.57 g, 2 mmol) in dry THF (40 mL) at 0 °C was added Et_3N (1 g, 10 mmol) and MsCl (1.14 g, 10 mmol). After stirring for 2 h, EtOH (5 mL) was added and solvent removed under reduced pressure and the residue redissolved in EtOAc (20 mL), filtered, evaporated and the residue purified by flash chromatography on silica gel (2 to 5% $MeOH/CH_2Cl_2$) to yield **29** as a colourless oil; yield: 392 mg (61%).

$C_{14}H_{22}NO_6PS$	calc.	C 46.26	H 6.06	N 3.85	P 8.54
(363.1)	found	46.41	5.92	3.91	8.19

MS (DCI): m/z = 364 (M + 1).

^{1}H NMR ($CDCl_3$): 1.25 (t, 3H), 2.0–1.8 (m, 4H, CH_2CH_2P), 3.01 (s, 3H, CH_3SO), 3.42 (dt, 2H, CH_2NHCO), 4.11 (dq, 2H, CH_2O), 4.21 (ddd, 2H, CH_2OMs), 7.19 (br, t, 1H, NHCO), 7.44–7.38 (m, 3H), 7.76 (dd, 2H, *o*-Ar)

1-{[(3-Benzamidopropyl)ethoxyphosphoryl]methyl}-1,4,7,10-tetraazacyclododecane (31):
To a solution of 1,4,7,10-tetraazacyclododecane (**2**, 0.16 g, 0.92 mmol) in dry DMF (20 mL) at 60 °C was added K_2CO_3 (0.13 g, 0.92 mmol) and a solution of the mesylate **29** (0.167 g, 0.46 mmol) in DMF (15 mL) over a period of 4 h under nitrogen. After 64 h, HPLC analysis (CM 300 cation exchange) revealed that the reaction was not progressing further, solvent was removed under reduced pressure and the residue redissolved in CH_2Cl_2 (10 mL). Filtration and evaporation yielded a residue which was purified by cation-exchange HPLC to yield **30** as a colourless gummy solid, t_R = 8.2 min (CM 300), yield: 52 mg (25%).

MS (DCI): m/z = 440 (M + 1).

^{1}H NMR ($CDCl_3$): δ = 1.30 (t, 3H, J = 7.6 Hz, CH_3), 1.97 (m, 5H, CH_2C + NH), 2.64–2.94 (m, 20H, $CH_2N + CH_2P$), 3.55 (dt, 2H, CH_2NHCO), 4.06 (dq, 2H, CH_2O), 7.38–7.47 (m, 3H), 7.93 (dd, 2H, *o*-Ar), 8.55 (t, 1H, NHCO).

1-{[(3-Benzamidopropyl)ethoyphosphoryl]methyl}-1,4,7-triazacyclononane (30):
This was prepared starting from **1** as described for the preparation of **31**; t_R = 6.8 min (CM 300); yield 28%.

MS (DCI): m/z = 398 (M + 2), 397 (M + 1), 256, 142.

^{1}H NMR ($CDCl_3$): δ = 1.56 (t, 3H, J = 6.8 Hz, CH_3), 2.26 (m, 2H, CH_2CH_2P), 2.88–3.18 (m, 14H, $CH_2N + CH_2PO$), 3.22–3.25 (ddd, 1H + 1H, J = 5.2 Hz, NCH_2PO), 3.75 + 3.88 (ddt, 1H + 1H, J = 6.4 Hz, CH_2NHCO), 4.33 (dq, 2H, CH_2O), 5.49 (br s, 2H, NH), 7.65–7.71 (m, 3H), 8.24–8.27 (dd, 2H, *o*-Ar), 9.05 (br t, 1H, NHCO).

(+)-(2*S*)-2-{4-[-(*p*-Nitrophenoxycarbonyl)propionamido]butyl}-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayltetrakis[methylene-(methylphosphinic Acid)] (22); Typical Procedure:
To a solution of the amine **19** (65 mg, 0.08 mmol) in dry DMSO (2 mL), *N*-methylmorpholine (74 mg, 0.73 mmol) was added followed by a solution of bis(*p*-nitrophenyl) succinate (57 mg, 0.16 mmol) in DMSO (2 mL) and the mixture was heated until solution occurred. After stirring for 4 h at 45 °C, HPLC analysis revealed completion of reaction, solvent was removed under reduced pressure and the residue purified by reverse-phase HPLC (Dynamax C-18, 60 Å; A = 0.1% TFA/H_2O, C = 0.1% TFA/MeCN; t = 0.90% A, 10% C; t = 20 min A = 25%, C = 75%; λ = 278 nm), t_R = 12.7 min. The product was isolated by lyophilisation as the tris(trifluoroacetate) salt; yield: 26 mg (38%).

MS (FAB): m/z = 833 (M + 1), 855 (M + Na).

^{1}H NMR (D_2O): δ = 1.45 (m, 13H, $CH_3P + CH_2C$), 1.70–1.90 (m, 2H, CH_2C), 2.65 (t, 2H, CH_2CO), 2.94 (t, 2H, CH_2CO), 3.10–3.85 (m, 25H, $CH_2N + CH_2P + CH_2NHCO$ + CHN), 7.35 (d, 2H), 8.32 (d, 2H).

The following compounds were prepared in a similar manner:

(+)-(2S)-2-{4-[3-(p-Nitrophenoxycarbonyl)propionamido]butyl}-1,4,7-triazacyclononane-1,4,7-triyltris[methylene(methylphosphinic Acid)] **(20)**: *yield 41%;* t_R = 12.1 min (Dynamax C_{18}-60 Å).

MS (FAB): m/z = 700 (M + 1), 699 (M).

^{1}H NMR (D_2O): δ = 1.25 (d + d + d, 9H, CH_3P), 1.8–1.5 (m, 6H, CH_2C), 2.68 (t, 2H, CH_2CO), 2.90–3.80 (m, 19H, $CH_2P + CH_2N$ + CHN), 7.38 (d, 2H), 8.34 (d, 2H).

1-{3-[3-(p-Nitrophenoxycarbonyl)propionamido]propylphosphinicomethyl}-1,4,7,10-tetraazacyclododecane-4,7,10-triyltris[methylene(phosphinic Acid)] **(36)**: yield 39%; t_R = 12.7 min (Dynamax C_{18}-60 Å).

MS (FAB): m/z = 805 (M + 1).

^{1}H NMR (D_2O): δ = 1.31 (d, 9H, CH_3P), 1.63 (br m, 4H, CH_2CH_2P), 2.55 (t, 2H, CH_2CO), 2.79 (t, 2H, CH_2CO), 3.05–3.50 (br m, 6H, $CH_2N + CH_2P + CH_2NHCO$), 7.28 (d, 2H), 8.19 (d, 2H)

(+)-(2S)-2-[4-Maleimidopropionamido)butyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayltetrakis[methylene(methylphosphinic Acid)] **(23)**:

Prepared using *N*-hydroxysuccinimidyl 3-maleimidopropionate instead of bis(*p*-nitrophenyl) succinate: yield 63%, colourless solid, t_R = 8.3 min (Dynamax C_{18}-60 Å)

MS (FAB): m/z = 763 (M + 1).

^{1}H NMR (D_2O): δ = 1.23 (d + d + br m, 16H, $CH_3P + CH_2CH_2C$), 1.61 (br m, 2H, CH_2C), 2.29 (t, 2H, CH_2CO), 2.60–3.90 (m, 27H, $CH_2N + CH_2P$ + CHN), 6.64 (s, 2H, CH).

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