Synthesis and Absolute Stereochemistry of the Two Diastereoisomers of P^3-1-(2-Nitrophenyl)ethyl Adenosine Triphosphate ('Caged' ATP)

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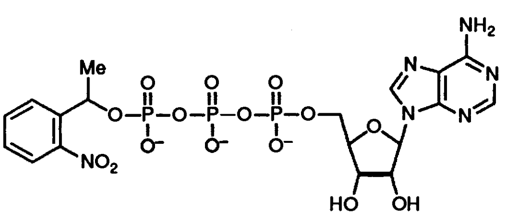
1-(2-Nitrophenyl)ethanol was resolved by fractional crystallisation of its diastereoisomeric (1*S*)-camphanates. The absolute stereochemistry of the (*S*)-alcohol was determined using the Horeau kinetic resolution procedure, and subsequently confirmed by X-ray crystallography of its (1*S*)-camphanate ester. The resolved alcohols were converted to (*R*)- and (*S*)-1-(2-nitrophenyl)ethyl phosphates, each of which was condensed with adenosine diphosphate to give the (*R*)- and (*S*)-1-(2-nitrophenyl)ethyl P^3-esters of adenosine triphosphate.

Since their introduction[1] in 1978, 1-(2-nitrophenyl)ethyl esters of inorganic phosphate and of adenosine triphosphate (ATP), together with related compounds have become widely used in biological research. They are commonly referred to as 'caged compounds' since they are generally inert, or nearly so, in biological systems until exposed to near ultraviolet light. In a process analogous to photoenolisation of ketones,[2] irradiation produces initially an *aci*-nitro intermediate which in a series of more or less well understood dark reactions decays to 2-nitrosoacetophenone and inorganic phosphate or ATP, (for a review of the known mechanistic information see ref. 3). The combined properties of biological inertness and photolytic sensitivity make these compounds useful in the study of rapid biological processes, since they may be diffused uniformly into a tissue preparation, whereafter flash photolysis rapidly releases the effector molecule (*e.g.* ATP) and permits real-time monitoring of biological events.[4] The presence of an asymmetric centre in the 1-(2-nitrophenyl)ethyl group implies that when this moiety esterifies the terminal phosphate of ATP, the resulting caged ATP **1** will contain two diastereoisomers. These can be at



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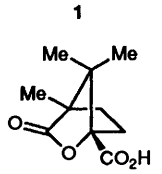

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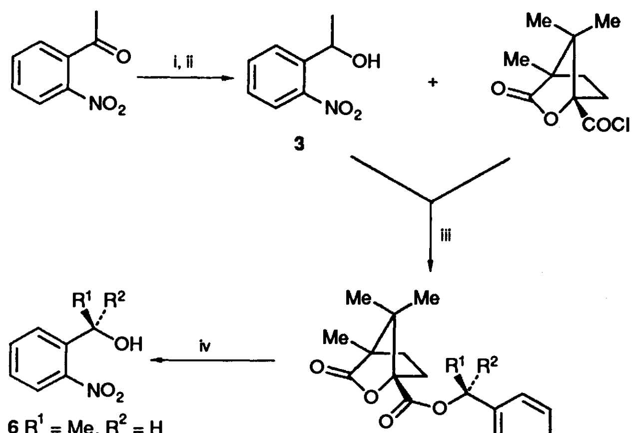
least partly separated by reverse-phase HPLC[5] and in a recent preliminary communication[6] it was suggested that the two diastereoisomers may show differential (weak) binding properties in muscle fibres. In order further to characterise this interaction, we needed to have available adequate quantities of the two diastereoisomers of caged ATP, with known absolute stereochemistry. Recent work by Schlichting *et al.*[7] on the preparation of crystalline complexes of the analogous caged guanosine triphosphate with p21*ras* proteins and subsequent time-resolved X-ray crystallography further illustrates the timeliness of this endeavour.

(1*S*)-Camphanic acid **2** is a valuable resolving agent for alcohols by virtue of its commercial availability, facile esterification *via* its acid chloride and presence of quaternary methyl groups which often show clear chemical shift differences in ^{1}H NMR spectra of derived diastereoisomeric camphanates and hence offer a convenient means to monitor the resolution process.[8,9] Sodium borohydride reduction of commercial 2-nitroacetophenone yielded racemic 1-(2-nitrophenyl)ethanol **3** containing approximately 2% of an impurity which appeared to be methyl 2-nitrobenzoate and communication with the supplier confirmed this as a probable contaminant.[10] Brief saponification of the crude material yielded the pure alcohol **3**, which was converted to the mixed diastereoisomeric (1*S*)-camphanates **4** and **5** as shown in Scheme 1. The ^{1}H NMR spectrum (90 MHz) of this mixture clearly showed six distinct resonances for methyl groups on quaternary carbons (*i.e.* three from each diastereoisomer) while the methyl group on the secondary carbon appeared as two barely distinct doublets.

Fractional crystallisation of the mixed camphanates from warm methanol (see Experimental section) readily yielded material heavily enriched in one isomer, subsequently shown to be the ester **4** derived from the (*R*)-alcohol, while the diastereoisomeric ester **5** was obtained after repeated crystallisation from isopropyl alcohol of the material in the mother liquor. After recycling the combined mother liquors from all crystallisations, the overall recovery of the separated diastereoisomers was 71% with cross-contamination of one isomer by the other at <0.3%, as assessed by ^{1}H NMR spectroscopy. Unexpectedly, the specific rotations of the diastereoisomeric camphanates were almost identical in magnitude, although of opposite signs. Saponification of the separate camphanates **4** and **5** gave the (*R*)- and (*S*)-alcohols **6** and **7** respectively in near-quantitative yield. The magnitudes of the specific rotations of the enantiomers, measured in two different solvents, were identical within experimental error.

The absolute configuration of the (*S*)-alcohol **7** was established initially by the Horeau kinetic resolution technique.[11] After following the described protocol, the recovered 2-phenylbutyric acid was laevorotatory, and as the 2-nitrophenyl group certainly has a steric bulk greater than methyl, application of the Horeau rule[11] determines the absolute stereochemistry of the (*S*)-alcohol. Although this result was unambiguous (see Experimental section) we were concerned to demonstrate the stereochemistry beyond doubt and therefore undertook an



Scheme 1 *Reagents:* i, $NaBH_4$–EtOH; ii, NaOH–MeOH; iii, pyridine; iv, KOH–aq.MeOH; v, $Pr^i_2NP(OCH_2CH_2CN)_2$–1*H*-tetrazole; vi, 3-$ClC_6H_4CO_3H$; vii, NaOH–MeOH; viii, $Ba(OAc)_2$; ix, Dowex 50 (pyridinium form), then $(C_8H_{17})_3N$; x, carbonyl diimidazole; xi, adenosine diphosphate

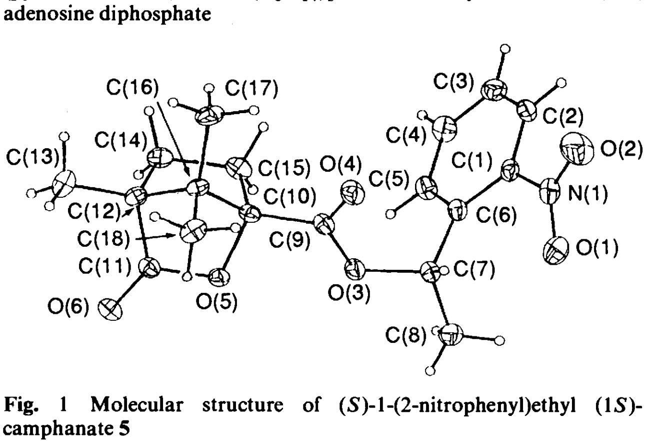


Fig. 1 Molecular structure of (*S*)-1-(2-nitrophenyl)ethyl (1*S*)-camphanate **5**

X-ray diffraction analysis of the related camphanate **5**. Fig. 1 shows the result of the structure determination which confirmed the stereochemistry already assigned. The absolute configuration was not determined experimentally but chosen on the basis of that for the camphanate fragment, which itself derives ultimately from the known absolute configuration of (+)-camphor.[12] Temperature factor coefficients, bond lengths and angles and F_o/F_c values from the X-ray crystallography are available from the Cambridge Crystallographic Data Centre.* The atomic coordinates are listed in Table 1.

Table 1 Fractional atomic co-ordinates ($\times 10^4$) for $C_{18}H_{21}NO_6$

	x	*y*	*z*
O(1)	1846(3)	−11849(2)	1592(5)
O(2)	3190(3)	−11190(3)	1274(6)
O(3)	−684(1)	−10708(1)	−365(3)
O(4)	307(2)	−9861(1)	977(4)
O(5)	−2204(1)	−9755(1)	−570(3)
O(6)	−3736(2)	−9239(1)	−878(4)
N(1)	2361(2)	−11410(2)	728(5)
C(1)	1993(2)	−11148(1)	−1036(4)
C(2)	2712(2)	−10938(2)	−2304(5)
C(3)	2417(3)	−10735(2)	−4012(5)
C(4)	1405(3)	−10738(2)	−4457(5)
C(5)	684(2)	−10932(2)	−3166(5)
C(6)	948(2)	−11136(2)	−1412(4)
C(7)	103(2)	−11268(2)	−37(4)
C(8)	−429(3)	−12010(2)	−295(7)
C(9)	−443(2)	−10022(2)	135(4)
C(10)	−1171(2)	−9455(1)	−600(4)
C(11)	−2830(2)	−9165(2)	−780(4)
C(12)	−2181(2)	−8476(1)	−917(4)
C(13)	−2737(3)	−7760(2)	−560(6)
C(14)	−1695(3)	−8577(2)	−2842(4)
C(15)	−985(2)	−9243(2)	−2613(4)
C(16)	−1298(2)	−8703(1)	371(4)
C(17)	−374(3)	−8192(2)	276(6)
C(18)	−1627(3)	−8784(2)	2367(4)

Racemic 1-(2-nitrophenyl)ethyl phosphate was previously prepared by phosphorylation of the alcohol **3** with anhydrous phosphoric acid and trichloroacetonitrile.[1] However the reaction proceeded only in modest yield and the conditions could risk racemising the optically active alcohols **6** and **7**. Accordingly we employed the alternative route shown in Scheme 1. Thus the (*R*)-alcohol **6** was subjected to phosphitylation[13] with *N*,*N*-diisopropyl bis(2-cyanoethyl)phosphoramidite followed by oxidation to the phosphotriester **8** with 3-chloroperbenzoic acid.[13] Both 2-cyanoethyl groups were removed by treatment with sodium hydroxide in warm methanol and the (*R*)-monophosphate was conveniently isolated as its barium salt **10**, in overall 60% yield from the alcohol **6**. The enantiomeric (*S*)-monophosphate was prepared as its barium salt **11** by an identical sequence from (*S*)-alcohol **7** and the two salts showed equal and opposite specific rotations.

Completion of the syntheses required coupling of the enantiomeric monophosphates with adenosine diphosphate to yield the individual diastereoisomers **12** and **13**. The ADP morpholidate procedure used previously[1] gave poor yields, but activation of the monophosphates with carbonyldiimidazole[14] followed by reaction with ADP proceeded smoothly. The coupling could also be achieved in similar yield by carbonyldiimidazole activation of ADP, followed by reaction with the caged monophosphate, but the protocol used avoids the need to hydrolyse the 2′,3′-*O*-cyclic carbonate formed when ADP is treated with carbonyldiimidazole.[15] as expected the individual diastereoisomers **12** and **13** each showed single symmetrical peaks with differing retention times on reverse-phase HPLC: the (*S*)-caged isomer **13** was the faster-eluting peak. Each diastereoisomer photolysed cleanly to release ATP (data not shown) and the two isomers have previously been shown to photolyse at identical rates.[16] Comparisons of the ^1H NMR spectra of the individual diastereoisomers revealed very

* For details of the C.C.D.C. deposition scheme, see *J. Chem. Soc., Perkin Trans. 1*, 1992, Issue 1, Instructions to Authors.

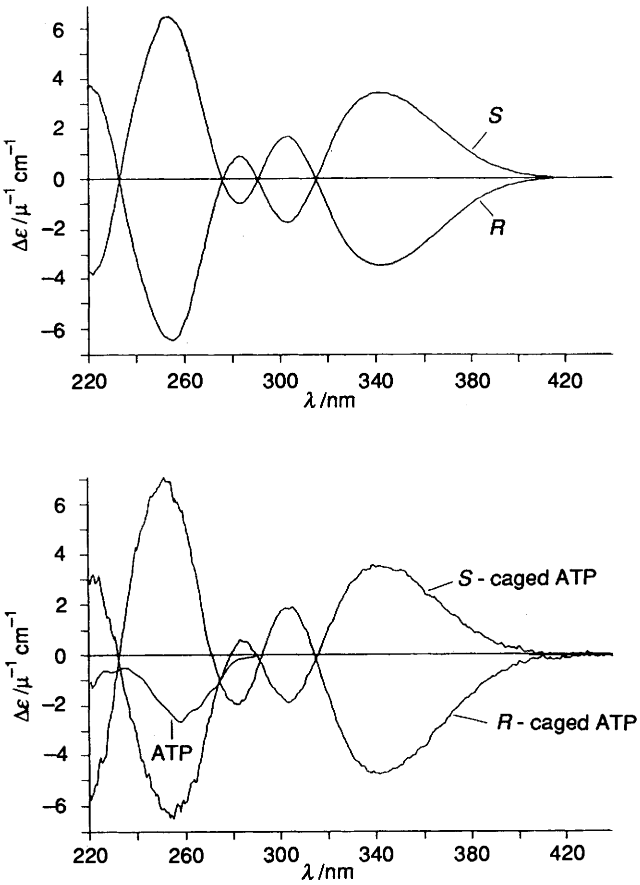


Fig. 2 CD spectra of (*R*)- and (*S*)-1-(2-nitrophenyl)ethyl phosphates **10** and **11** (upper panel), and of ATP and its (*R*)- and (*S*)-caged diastereoisomers **12** and **13** (lower panel)

minor differences, as has previously been reported in part for the benzylic proton in the mixed isomers.[16] In addition, slight differences in chemical shifts were apparent for one of the purine protons and for the anomeric proton on the ribose ring (see Experimental section). However, these differences would not be a reliable method for assignment of stereochemistry to future samples of these compounds and we have therefore recorded their CD spectra, which are shown in Fig. 2. For comparison the CD spectra of the (*R*)- and (*S*)-1-(2-nitrophenyl)ethyl phosphates **10** and **11** are also shown. The spectra of the latter two compounds show the expected mirror image relationship, and the spectra of the diastereoisomers **12** and **13** show clear relationships to those of their parent enantiomeric phosphates **10** and **11**.

This work provides ready access to the two separate diastereoisomers of caged ATP on a scale of several hundred micromoles, although the exact yields depend on the degree of purity required for subsequent biological experiments (see Experimental section). Details of the use of the individual diastereoisomers will be published elsewhere.

Experimental

Microanalyses were carried out by Butterworth Laboratories, Teddington, Middlesex. NMR spectra were determined on JEOL FX9OQ or Bruker WM200 spectrometers. *J* values are given in Hz. CD spectra were obtained on a Jasco J-600 spectropolarimeter for solutions in 10 mmol dm^{-3} sodium phosphate, pH 7.0. Phosphates were converted to their sodium salts by treatment with Dowex 50 (Na form) for NMR and CD spectroscopic studies. Negative ion FAB mass spectra were run on a VG 70-250SE instrument for samples in a glycerol matrix. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

(1*S*)-Camphanic acid was purchased from Aldrich Chemical Co., Gillingham, Dorset and had $[\alpha]_D^{25}$ −7.1 (*c* 2.03, 95% EtOH) (lit.,[17] $[\alpha]_D$ −9.3 in 95% EtOH).

(RS)-1-(2-*Nitrophenyl)ethanol* **3**.—A solution of 2-nitroacetophenone (8.26 g, 50 mmol) in ethanol (200 cm^3) was cooled in ice and $NaBH_4$ (1.89 g, 50 mmol) was added in one portion. The solution was stirred in the ice bath for 1 h then neutralised with glacial acetic acid (*ca.* 8 cm^3) and concentrated to a small volume under reduced pressure. The residue was diluted with ether (200 cm^3) and washed with water, saturated $NaHCO_3$ and brine and evaporated to leave a yellow oil, which showed *inter alia* a weak carbonyl absorption at ν/cm^{-1} 1745 and a ^1H NMR signal at δ_H 3.88. The latter signal was also present in the starting material and was assigned to the presence of methyl 2-nitrobenzoate as a contaminant. Therefore the crude product was dissolved in MeOH (150 cm^3) together with aq. KOH (2 mol dm^{-3}; 18 cm^3) and heated under reflux for 0.5 h, then cooled and worked up as above. Distillation of the residual material gave the pure alcohol **3** as a yellow oil (6.26 g, 75%), b.p. 106–107 °C (0.7 mmHg) [lit.,[1] b.p. 105 °C (1 mmHg)].

(R)- *and* (S)-1-(2-*Nitrophenyl)ethyl* (1S)-*Camphanates* **4** *and* **5**.—Crude (1*S*)-camphanic acid chloride [prepared from (1*S*)-camphanic acid monohydrate (10 g, 46 mmol) by the method of Gerlach[8]] was mixed with a solution of (*RS*)-1-(2-nitrophenyl)-ethanol (7.04 g, 42.1 mmol) in anhydrous pyridine (70 cm^3) and allowed to stand overnight at room temp. The solution was poured over ice (*ca.* 200 g) and the product was recovered by extraction with ethyl acetate (3 × 100 cm^3). The combined organic extracts were washed with water, 0.5 mol dm^{-3} HCl and saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated under reduced pressure to leave the diastereoisomeric camphanates as an off-white solid (14.0 g). The mixed isomers were dissolved in a minimum volume of boiling MeOH and the solution was allowed to cool to room temp., whereupon two crystal forms were evident. Long needles formed initially and became encrusted with small prisms as the solution cooled further. All crystals were redissolved by reheating the solvent and the solution was again allowed to cool. Immediately the small prisms began to form on the needles, the supernatant was rapidly decanted, and the needles were twice recrystallised from MeOH to give the pure (*R,S*)-ester **4**. The decanted supernatant was evaporated under reduced pressure and the residue crystallised eight times from isopropyl alcohol to give the pure (*S,S*)-ester **5**. Mother liquors from all crystallisations were combined and evaporated, and the residues taken through a further cycle of crystallisation as above. The overall recoveries were (*R,S*)-ester (4.90 g) and (*S,S*)-ester (5.45 g). The (R,S)-*ester* had m.p. 141–142 °C (Found: C, 62.05; H, 5.9; N, 4.2. $C_{18}H_{21}NO_6$ requires C, 62.2; H, 6.1; N, 4.0%); $[\alpha]_D^{25}$ −198.6 (*c* 1.01, $CHCl_3$); λ_{max} EtOH/nm 253 (ε/mol dm^{-3} cm^{-1} 5300); δ_H (90 MHz; $CDCl_3$; Me_4Si standard) 7.3–8.0 (m, 4 H, Ar-H), 6.52 (q, 1 H, *J* 6.5, ArC*H*), 1.7–2.6 (m, 4 H, CH_2), 1.71 (d, 3 H, CHCH_3), 1.10 (s, 3 H, Me), 1.02 (s, 3 H, Me) and 0.85 (s, 3 H, Me). The (S,S)-*ester* had m.p. 133–134 °C (Found: C, 62.1; H, 6.1; N, 4.2. $C_{18}H_{21}NO_6$ requires C, 62.2; H, 6.1; N, 4.0%); $[\alpha]_D^{25}$ +202.8 (*c*, 1.01, $CHCl_3$); δ_H (90 MHz; $CDCl_3$; Me_4Si standard) 7.3–8.0 (m, 4 H, Ar-H), 6.53 (q, 1 H, *J* 6.5, ArC*H*), 1.7–2.5 (m, 4 H, CH_2), 1.70 (d, 3 H, CHCH_3), 1.13 (s, 3 H, Me), 1.04 (s, 3 H, Me) and 1.00 (s, 3 H, Me).

(R)- *and* (S)-1-(2-*Nitrophenyl)ethanol* **6** *and* **7**.—The (*R,S*)-ester **4** (4.8 g, 13.8 mmol) was dissolved in a mixture of MeOH (75 cm^3) and KOH (2 mol dm^{-3}; 10 cm^3) and the solution was heated under reflux for 0.5 h, then cooled, neutralised with 2 mol dm^{-3} HCl and concentrated under reduced pressure. The residue was extracted with ether and the organic extract was washed with saturated $NaHCO_3$, dried (Na_2SO_4) and evaporated. The residue was purified by short-path distillation at 0.6 mmHg (Kugelrohr, oven temperature 190 °C) to yield (*R*)-1-(2-nitrophenyl)ethanol **6** (2.17 g, 13.0 mmol) as a pale yellow liquid which crystallised to a pale solid, m.p. 60–61 °C on storage at 4 °C; $[\alpha]_D^{27}$ −54.0 (*c* 0.97, $CHCl_3$), $[\alpha]_D^{25}$ −212.0 (*c* 1.01, HOAc). The (*S*)-enantiomer **7** was prepared in an identical manner and gave a pale solid, m.p. 60–61 °C on storage at 4 °C; $[\alpha]_D^{27}$ +54.2 (*c* 0.98, $CHCl_3$), $[\alpha]_D^{25}$ +215.7 (*c* 1.01, HOAc). The ^1H NMR spectrum of each enantiomer was identical to that of the racemic alcohol.

Absolute Configuration of (S)-1-(2-*Nitrophenyl*)*ethanol*.—A portion of the (*S*)-alcohol **7** (19.1 mg, 0.114 mmol) was treated with (±)-2-phenylbutyric anhydride in pyridine according to the method of Horeau and Kagan.[11] Alkaline titration of the excess 2-phenylbutyric acid as described[11] indicated an esterification yield of 91.2%. The recovered 2-phenylbutyric acid had $[\alpha]_D^{25}$ −14.2 (*c* 2.83, benzene) which corresponded to an optical yield of 48.7%.

X-Ray Crystallography of (S)-1-(2-*Nitrophenyl*)*ethyl* (1S)-*Camphanate* **5**.—Crystallographic measurements were made on a crystal of dimensions 0.5 × 0.25 × 0.1 mm^3 mounted in air on a glass capillary. Following preliminary photography, the unit cell dimensions were obtained and intensity data recorded using a CAD4 diffractometer operating in the $\omega/2\theta$ scan mode with Ni-filtered Cu-Kα radiation, in a manner described previously.[18] The structure was solved by direct methods and refined by full-matrix least squares, with non-hydrogen atoms treated anisotropically and hydrogen atoms isotropically.

Crystal data. $C_{18}H_{21}NO_6$, $M = 347.37$. Orthorhombic, $a = 13.147(1)$, $b = 18.045(3)$, $c = 7.331(1)$ Å, $V = 1\ 739.2$ Å^3, space group $P2_12_12_1$, $Z = 4$, $D_c = 1.327$ g cm^{-3}, $F(000) = 736$, $\lambda = 1.54178$ Å.

Data collection. $2.0 \leqslant \theta \leqslant 70.0°$, $T = 293$ K, 1973 intensities measured, 1900 unique, 1608 observed $[F_o > 3(F_o)]$. Empirical absorption correction. Refinement: 245 parameters, weights = $[\sigma^2(F_o) + 0.015\ F_o{}^2]$, $R = 0.041$, $R_w = 0.068$.

Barium (R)- *and* (S)-1-(2-*Nitrophenyl*)*ethyl Phosphate* **10** *and* **11**.—*N*,*N*-Diisopropyl bis(2-cyanoethyl)phosphoramidite[19] was prepared from bisisopropylphosphoramidous dichloride[20] and purified as described.[21] A solution of (*R*)-1-(2-nitrophenyl)ethanol (334 mg, 2 mmol) and the phosphoramidite reagent (677 mg, 2.5 mmol) in dry THF (15 cm^3) was treated with 1*H*-tetrazole (314 mg, 5.5 mmol) and stirred under N_2 for 1 h at room temp., then cooled in ice and treated dropwise over 5 min with a solution of 3-chloroperbenzoic acid (61% per-acid; 940 mg, 3.33 mmol) in dichloromethane (10 cm^3). The mixture was stirred on ice for 0.5 h and for a further 0.5 h at room temp., then diluted with ether and washed successively with 10% $Na_2S_2O_5$, 1 mol dm^{-3} HCl, 5% $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated under reduced pressure. The semi-solid residue was dissolved in 0.1 mol dm^{-3} methanolic NaOH (40 cm^3), kept at 50 °C for 0.5 h and concentrated under reduced pressure. The residue was partitioned between water and ether (each 80 cm^3) and the aqueous layer was adjusted to pH 7 with 1 mol dm^{-3} acetic acid. A solution of $Ba(OAc)_2$ (2 mol dm^{-3}; 7 cm^3) was added and the aqueous solution was concentrated under reduced pressure to *ca.* 30 cm^3. Ethanol (20 cm^3) was added with stirring and the solution was allowed to stand at 4 °C for several hours. The precipitate was filtered, washed successively with 1:1 EtOH–H_2O, EtOH and ether and dried under reduced pressure to give barium (*R*)-1-(2-nitrophenyl)ethyl phosphate **10** as pale cream plates (454 mg, 60%); $[\alpha]_D^{25}$ −120 (*c* 1.0, HOAc). The (*S*)-isomer, **11** prepared in an identical manner, had $[\alpha]_D^{25}$ +117 (*c* 1.0, HOAc).

(R)- *and* (S)-P^3-1-(2-*Nitrophenyl*)*ethyl Adenosine Triphosphate* **12** *and* **13**.—Dowex 50 (H^+ form) (10 g dry weight) was converted to its pyridinium form by sequential washes of 1 mol dm^{-3} HCl, water, 20% aqueous pyridine, and again of water. The resin was resuspended in water (10 cm^3), barium (*R*)-1-(2-nitrophenyl)ethyl phosphate **10** (382 mg, 1 mmol) was added and the mixture was stirred gently until the barium salt dissolved. The resin was filtered and washed well with water, and the combined filtrate and washings were mixed with tri-n-octylamine (353 mg, 1 mmol). The water was removed by rotary evaporation (oil pump for all evaporations) and the residue was dried by sequential cycles of dissolution in, and evaporation of, dry pyridine (50 cm^3) and dry DMF (4 × 50 cm^3). The dried residue was redissolved in dry DMF (2 cm^3), treated with carbonyl diimidazole (0.81 g, 5 mmol) and stirred at room temp. for 6 h. Dry methanol (0.25 cm^3, 6.25 mmol) was added and the mixture kept for 45 min, then evaporated to dryness under reduced pressure.

Meanwhile, adenosine diphosphate triethylammonium salt (1.2 mmol) was converted to its pyridinium salt with Dowex resin as described above and the aqueous filtrate was treated with trioctylamine (847 mg, 2.4 mmol). The mixture was further processed by evaporations from pyridine and DMF as above and finally dissolved in dry DMF (10 cm^3). This solution was added to the activated phosphate prepared above and the solvent was removed by rotary evaporation. The residue was dissolved in dry hexamethylphosphoric triamide (3 cm^3) and the resulting solution stirred at room temp. for 4 d, then mixed with water (25 cm^3) and extracted with $CHCl_3$ (4 × 50 cm^3). The aqueous phase was evaporated to dryness and redissolved in water (3 cm^3). Analytical HPLC [Waters μBondapak C_{18} column; mobile phase 10 mmol dm^{-3} KH_2PO_4, pH 6.5 plus 5% (v/v) acetonitrile, flow rate 1.5 cm^3 min^{-1}] showed the product **12**, t_R 23.5 min, in a crude yield of 40–50%. For purification, the crude material was chromatographed on a preparative C_{18} reversed-phase HPLC column (2 × 30 cm) and initially eluted with 50 mmol dm^{-3} NaOAc (adjusted to pH 6.5 with NaOH) plus 5% (v/v) methanol until no further ADP emerged. The methanol content of the eluting buffer was then increased to 15% to elute the (*R*)-caged ATP **12**. When complete separation from traces of ADP (<0.02%) was required, the leading half of the caged ATP peak was discarded and the trailing half was desalted as previously described.[16] The yield of this rigorously purified product was 10–15% (Found: M^- 655. $C_{18}H_{20}N_6O_{15}P_3$ + 2H requires *M* 655); δ_H (200 MHz; D_2O pH 7; Me_2CO standard) 8.45 (s, 1 H, purine-H), 8.20 (s, 1 H, purine-H), 7.35–7.95 (m, 4 H, Ar-H), 6.05 (d, 1 H, $J_{1',2'}$ 4.9, 1′-H), 5.89 (m, 1 H, $J_{H,P}$ 7.9, ArCH), 4.51 (m, 1 H, 2′-H), 4.32 (m, 1 H, 3′-H), 4.18 (br s, 1 H, 4′-H) and 1.51 (d, 3 H, *J* 6.9, CH_3). The 5′-H signal was obscured by the HOD peak. The proton–phosphorus coupling of the benzylic proton was measured after irradiation of the adjacent methyl group to remove the vicinal proton–proton coupling.

The (*S*)-caged isomer **13**, prepared in an identical manner from barium (*S*)-1-(2-nitrophenyl)ethyl phosphate **11**, had t_R 21.0 min in analytical HPLC as described above (Found: M^- 655. $C_{18}H_{20}N_6O_{15}P_3$ + 2H requires *M* 655); δ_H(200 MHz; D_2O pH 7, Me_2CO standard) 8.43 (s, 1 H, purine-H), 8.20 (s, 1 H, purine-H), 7.35–7.95 (m, 4 H, Ar-H), 6.04 (d, 1 H, $J_{1',2'}$ 5.9, 1′-H), 5.88 (m, 1 H, $J_{H,P}$ 7.9, ArCH), 4.51 (m, 1 H, 2′-H), 4.34 (m, 1 H, 3′-H), 4.19 (br s, 1 H, 4′-H) and 1.52 (d, 3 H, *J* 6.9, CH_3). Proton–phosphorus coupling was measured as for the (*R*)-caged isomer. The 5′-H signal was obscured by the HOD peak. Separate co-injections of the individual diastereoisomers **12** and **13** together with (*RS*)-caged ATP under the analytical HPLC conditions described above specifically augmented one or other of the two partially resolved peaks present in the (*RS*)-compound alone, according to which diastereoisomer was coinjected.

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