ethanol at 21 °C. After an additional 16 h at 21 °C, the solution was heated at reflux for 2 h. The solvent was then evaporated and the residue was distilled on a Kugelrohr apparatus at 190 °C (1 torr). The orange distillate was dissolved in ethyl acetate and the resulting solution washed with 10% HCl, saturated $NaHCO_3$, and brine. The organic solution was dried over sodium sulfate, concentrated, and recrystallized from ethyl acetate/hexane to give 12.2 g (67%) of **9**: mp 82–86 °C; ^1H NMR (60 MHz) δ 4.65 (q, J = 7 Hz, 2 H), 4.12 (s, 3 H), 4.10 (s, 3 H), 1.51 (t, J = 7 Hz, 3 H); ^{13}C NMR (22.62 MHz) δ 162.88, 161.25, 156.96, 149.74, 149.15, 64.04, 54.29, 14.17; IR 3000, 2970, 1740, 1450, 1390, 1290, 1220, 1185, 1100, 1020, 980; MS 269 (M^+), 238, 224, 211, 183, 111 (base). Anal. Calcd for $C_{10}H_{11}N_3O_6$: C, 44.62; H, 4.12; N, 15.61. Found: C, 44.72; H, 4.09; N, 15.54.

6-(Carboethoxy)-2,3-bis(carbomethoxy)-5-methyl-4-phenylpyridine (10) and 6-(Carboethoxy)-2,3-bis(carbomethoxy)-4-methyl-5-phenylpyridine (11). A mixture of 4.05 g (15.0 mmol) of triazine **9** and 6.0 mL (48 mmol) of 1-phenylpropyne was heated in a Parr bomb at 200 °C for 12 h. The resulting product was chromatographed (3:7 ethyl acetate/hexane) to give 3.35 g (62%) of a 3:2 mixture of pyridines **10** and **11**, respectively. Crystallization from ethyl acetate/hexane afforded 0.95 g (18%) of **10**: mp 91.5–92.5 °C; ^1H NMR (60 MHz) δ 7.6–7.1 (m, 5 H), 4.51 (q, J = 7 Hz, 2 H), 3.98 (s, 3 H), 3.56 (s, 3 H), 2.27 (s, 3 H), 1.43 (t, J = 7 Hz, 3 H); ^{13}C NMR (75.473 MHz) δ 166.75, 165.82, 164.60, 150.65, 150.35, 141.72, 135.19, 134.92, 133.27, 128.81, 128.50, 128.39 62.35, 53.33, 52.46, 16.79, 14.18; IR 3010, 2960, 1745, 1730, 1720, 1445, 1375, 1350, 1235, 1160, 1105, 1015; MS 357 (M^+), 327, 326, 314, 299, 286, 254 (base). Anal. Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.68; H, 5.34; N, 3.95. Further purification of the mother liquor by column chromatography with the above solvent system followed by crystallization of selected fractions furnished by 0.17 g (3%) of **11**: mp 90–91 °C (ethyl acetate/hexane); ^1H NMR (60 MHz) δ 7.6–7.1 (m, 5 H), 4.12 (q, J = 7 Hz, 2 H), 4.05 (s, 6 H), 2.23 (s, 3 H), 0.99 (t, J = 7 Hz); ^{13}C NMR (75.473 MHz) δ 167.57, 165.61, 164.74, 150.56, 145.96, 143.29, 139.38, 135.19, 128.57, 128.38, 61.79, 53.39, 53.08, 17.25, 13.55; IR 2990, 2960, 1730, 1445, 1350, 1295, 1225, 1200, 1085, 1030; MS 357 (M^+), 327, 326, 313, 298, 285, 284 (base), 251, 241, 195, 167. Anal. Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.60; H, 5.41; N, 3.86.

5-Methyl-4-phenyl-2,3,6-tricarboxypyridine (12). The triester **10** (0.829 g, 2.32 mmol) was added to a magnetically stirred solution of 2.9 g (22 mmol) of aluminum chloride in 25 mL of ethanethiol at 0 °C. After 2 h the solution was taken up in ethyl acetate and washed with 10% HCl and then brine. The organic solution was dried over Na_2SO_4 and concentrated and the residue recrystallized from ethyl acetate/hexane to give 0.533 g (76%) of **12**: mp 196–197 °C; ^1H NMR (Me_2SO-d_6, 60 MHz) δ 7.6–7.1 (m, 5 H), 2.16 (s, 3 H); ^{13}C NMR (Me_2SO-d_6, 22.62 MHz) δ 167.03, 165.54, 150.19, 149.28, 142.52, 135.30, 133.03, 132.77, 128.41, 16.19; IR 3450 (br), 1730, 1700, 1445, 1255, 1190, 1110; MS 283 (M^+ – H_2O), 256, 239, 210, 44 (base). Anal. Calcd for $C_{15}H_{11}NO_6$: C, 59.80; H, 3.68; N, 4.65. Found: C, 59.66; H, 3.69; N, 4.62.

3-Carboxy-2,6-bis(carbomethoxy)-5-methyl-4-phenylpyridine (13). The triacid **12** (533 mg, 1.77 mmol) was treated with 100 mL of 10% methanolic HCl for 18 h at 21 °C. The solution was then concentrated, and the residue was recrystallized from ethyl acetate/hexane to give 525 mg (90%) of **13**: mp 155–157 °C; ^1H NMR (300 MHz) δ 7.42 (m, 3 H), 7.19 (m, 2 H), 4.00 (s, 3 H), 3.96 (s, 3 H), 2.28 (s, 3 H); ^{13}C NMR (75.473 MHz) δ 169.13, 166.00, 164.44, 150.68, 149.48, 141.69, 136.03, 134.76, 132.73, 128.84, 128.56, 128.50, 53.33, 53.11, 16.91; IR 3400 (br), 2950, 1730, 1445, 1355, 1230, 1105; MS 329 (M^+), 328, 296, 284, 270, 253, 239, 227 (base), 194, 166. Anal. Calcd for $C_{17}H_{15}NO_6$: C, 62.00; H, 4.59; N, 4.25. Found: C, 62.00; H, 4.62; N, 4.20.

3-Amino-2,6-bis(carbomethoxy)-5-methyl-4-phenylpyridine (14). A solution of 525 mg (1.60 mmol) of acid **13**, 0.50 g (1.8 mmol) of diphenylphosphoryl azide, and 0.50 mL (3.6 mmol) of triethylamine in 30 mL of benzene was heated at reflux for 1 h, whereupon 2 mL of water was added and the heating continued for 45 min. The solution was then concentrated. A solution of the residue in ethyl acetate was washed with water and then brine, dried over Na_2SO_4, and concentrated. The product was recrystallized from ethyl acetate/hexane to yield 287 mg (60%) of **14**: mp 179–181 °C; ^1H NMR (300 MHz) δ 7.53 (t, J = 8 Hz, 2 H), 7.44 (t, J = 8 Hz, 1 H), 7.17 (d, J = 8 Hz, 2 H), 6.2–5.8 (br s, 2 H), 3.96 (s, 3 H) 3.92 (s, 3 H), 2.26 (s, 3 H); ^{13}C NMR (75.473 MHz) δ 167.61, 166.14, 146.56, 138.64, 137.32, 136.22, 134.31, 129.92, 129.07, 128.86, 124.90, 52.67, 52.49, 17.87; IR 3480, 3360, 3060, 3000, 2950, 1710, 1685, 1585, 1435, 1350, 1310, 1245, 1205, 1105; MS 300 (M^+), 285, 242, 210 (base), 182. Anal. Calcd for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.93; H, 5.46; N, 9.43.

Acknowledgment. I appreciate the assistance of Dr. M. L. Maddox and Mrs. J. Nelson in the obtaining and interpreting of the NMR spectra.

Pyridine Construction via Thermal Cycloaddition of 1,2,4-Triazines with Enamines: Studies on the Preparation of the Biaryl CD Rings of Streptonigrin

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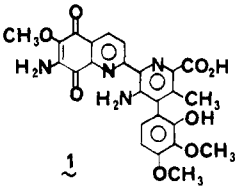
Streptonigrin (1), an antitumor antibiotic isolated from



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cultures of *Streptomyces flocculus*, has been the subject of extensive biological and chemical studies.[2] Its antibiotic activity against gram-positive and gram-negative bacteria and its potent antitumor activity have provided the incentive for much synthetic work[2] which has culminated recently in two separate reports of its total synthesis.[3] Herein we disclose the results of an initial investigation on the development of a convergent approach to streptonigrin based on our recent observation that 1,2,4-triazines undergo a regiospecific, inverse electron demand Diels–Alder reaction with pyrrolidine enamines to afford substituted pyridines.

A key to the synthesis of the streptonigrin carbon framework lies in the preparation of a pentasubstituted pyridine (e.g., **2**),[3] and our studies on the utility of the

(1) Chicago Community Trust Co./Searle Scholar Recipient, 1981–1984.

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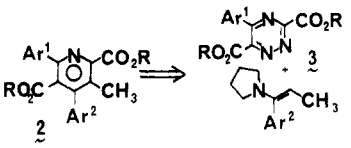
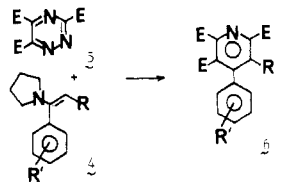
0022-3263/82/1947-3763$01.25/0

Scheme I



cycloaddition reaction of pyrrolidine enamines with 1,2,4-triazines[4] suggested that this reaction could provide an effective method for the regiospecific construction of the pyridyl biaryl CD ring system of streptonigrin (Scheme I). To test the feasibility of this approach, we have investigated the reaction of pyrrolidine enamines **4** with 3,5,6-tricarbethoxy-1,2,4-triazine (**5**,[5] eq 1), and the results of this study are detailed below.



(1)

a R=R'= H
b R= CH_3, R'= H
c R= CH_3, R'= 2-OCH_2Ph-3,4-$(OCH_3)_2$
E= $CO_2CH_2CH_3$

In each case, the initial cycloaddition reaction is *exothermic* and is accompanied by the immediate evolution of nitrogen. In previous studies we have noted that the cycloaddition reaction of 1,2,4-triazine with pyrrolidine enamines derived from aliphatic or cyclic ketones is often exothermic, yet attempts to induce the pyrrolidine enamine of acetophenone to react with 1,2,4-triazine have failed.[4] The exothermic reaction observed for the reaction of enamines **4a–c** with **5** can be attributed to the enhanced electrophilicity of the 1,2,4-triazine nucleus imparted by the carboethoxy substituents. The biaryl pyridine products **6a–c** were formed predominately, if not exclusively, and no material arising from alternative cycloaddition products,[6] little or no material derived from cycloaddition with the reversed regioselectivity,[6] and no material resulting exclusively from potential dipolar intermediates could be detected. The pyrrolidine enamine of acetophenone (**4a**) afforded the 4-phenyl-substituted pyridine **6a** exclusively ($CHCl_3$, 45 °C, 24 h, 45%), and no trace (<2%) of isomeric products could be detected chromatographically or spectroscopically. The pyrrolidine enamine **4b** afforded **6b** predominately (>15:1, $CHCl_3$, 45 °C, 8 h, 73%), and only traces of an isomeric product (<6%, presumably the 4-methyl-3-phenyl-substituted pyridine) could be detected spectroscopically. The biaryl product **6c** derived from the pyrrolidine enamine of 2-(benzyloxy)-3,4-dimethoxypropiophenone, **4c**, was constructed under *unusually mild conditions* ($CHCl_3$, 45 °C, 3 h, 59%, >9.1), using this process, thus confirming the ease and potential versatility of this approach to streptonigrin (**1**) and related compounds. Preliminary evidence for the structural assignments was derived from carbon-13 NMR data on **6a–c**, and conclusive proof for the assignments rest on the fact that decarboxylation[7] of **6a,b** gave the known 4-phenylpyridine (45%) and 3-methyl-4-phenylpyridine, respectively. The carbon-13 assignments for **6c** parallel closely that of streptonigrin.[1,8]

Efforts on the preparation of an appropriate 1,2,4-triazine (**3**), its application to the total synthesis of streptonigrin (**1**), and further application of this methodology to the total synthesis of related antitumor antibiotics[9] are the focus of current efforts and they will be reported in due course.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded with a Beckmann Model 33 or Perkin-Elmer Model 727 spectrophotometer. ^{13}C and ^1H NMR spectra were obtained on a Varian FT-80A spectrophotometer, and chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Electron-impact mass spectra were obtained with a Varian CH-5 spectrometer. Elemental analyses were performed on an HP-185C CHN analyzer by Mr. T. Nguyen at the University of Kansas and Microanalytical Laboratory.

4-Phenyl-2,3,6-tricarbethoxypyridine (6a). A solution of 3,5,6-tricarbethoxy-1,2,4-triazine[5] (126 mg, 0.5 mmol) in $CHCl_3$ (0.5 mL) was treated with α-pyrrolidinylstyrene (**4a**; 173 mg, 1.0 mmol, 2.0 equiv) in $CHCl_3$ (0.5 mL) under nitrogen (25 °C), and the resulting orange solution evolved nitrogen immediately. The mixture was stirred for 1.25 h (25 °C) and 22.75 h (45 °C). Chromatography of the crude product (SiO_2, 40% ether–pentane eluant) afforded 83 mg (186 mg theoretical, 45%) of pure **6a**: mp 99–100.5 °C (triturated three times from ether); ^1H NMR ($CDCl_3$) δ 8.25 (1 H, s, aromatic), 7.44 (5 H, s, Ph), 4.47 (2 H, q, J = 8 Hz, CH_2), 4.45 (2 H, q, J = 8 Hz, CH_2), 4.18 (2 H, q, J = 8 Hz, CH_2), 1.45 (3 H, t, J = 8 Hz, CH_3), 1.42 (3 H, t, J = 8 Hz, CH_3), 1.05 (3 H, t, J = 8 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.4 (C=O), 164.5 (C=O), 164.0 (C=O), 150.5 (C-2), 148.4 (C-6), 147.1 (C-4), 136.5 (C-3), 132.7 (C-1'), 129.4 (C-4'), 128.8 (C-3'), 128.2 (C-2'), 127.9 (C-5), 62.7 (CH_2), 62.5 (CH_2), 62.0 (CH_2), 14.3 (CH_3), 14.1 (CH_3), 13.6 (CH_3); IR ($CHCl_3$) ν_{max} 3010, 3000, 1730 (C=O), 1580, 1372, 1340, 1275, 1238, 1135, 1060, 1010, 895, 672 cm^{-1}; mass spectrum, m/e (relative intensity) 371 (M^+, 1), 328 (6), 327 (29), 299 (93), 298 (51), 253 (83), 227 (base), 225 (81), 224 (20), 181 (24), 180 (29), 155 (33), 153 (16), 152 (13), 128 (14), 53 (5). Anal. Calcd for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.56; H, 5.80; N, 3.50.

5-Methyl-4-phenyl-2,3,6-tricarbethoxypyridine (6b). A solution of 3,5,6-tricarbethoxy-1,2,4-triazine[5] (126 mg, 0.5 mmol) in $CHCl_3$ (0.5 mL) was treated with 1-pyrrolidino-1-phenyl-1-propene (**4b**; 188 mg, 1.0 mmol, 2.0 equiv) in $CHCl_3$ (0.5 mL) under nitrogen (25 °C), and the resulting orange solution evolved nitrogen immediately. The mixture was stirred for 1.25 h (25 °C) then 6.75 h (45 °C). Chromatography of the crude product (SiO_2, 25% ether–pentane eluant) afforded 140 mg (193 mg theoretical, 73%) of pure **6b**: mp 95–96 °C (triturated three times from ether); ^1H NMR ($CDCl_3$) δ 7.47–7.19 (5 H, m, Ph), 4.47 (2 H, q, J = 8 Hz, CH_2); 4.45 (2 H, q, J = 8 Hz, CH_2), 4.02 (2 H, q, J = 8 Hz, CH_2), 2.25 (3 H, s, $ArCH_3$), 1.43 (3 H, t, J = 8 Hz, CH_3), 1.40 (3 H, t, J = 8 Hz, CH_3), 0.94 (3 H, t, J = 8 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.2 (C=O), 165.9 (C=O), 164.3 (C=O), 150.5 (C-2), 150.4 (C-6), 147.0 (C-4), 135.2 (C-3), 134.6 (C-5), 133.0 (C-1'), 128.7 (C-4'), 128.6 (C-3'), 128.4 (C-2'), 62.4 (CH_2), 62.2 (CH_2), 61.6 (CH_2), 16.6 ($ArCH_3$), 14.1 (CH_3), 13.6 (CH_3), 13.3 (CH_3); IR ($CHCl_3$) ν_{max} 3010, 2995, 1720 (C=O), 1548, 1428, 1358, 1322, 1270, 1232, 1200, 1155, 1132, 1080, 995, 835, 673 cm^{-1}; mass spectrum, m/e (relative intensity) 385 (M^+, 13), 384 (22), 341 (33), 340 (30), 314 (19), 313 (94), 312 (57), 269 (13), 268 (71), 241 (base), 240 (47), 239 (71),

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(5) Rätz, R.; Schroeder, H. *J. Org. Chem.* **1958**, *23*, 1931.
(6) In recent studies we have observed that electron-withdrawing groups on the 1,2,4-triazine nucleus can have a profound effect on the observed regioselectivity in the cycloaddition reaction with pyrrolidine enamines. A detailed investigation of factors influencing or governing the mode of cycloaddition and the observed regioselectivity is currently in progress and will be the subject of future work.
(7) Structures **6a** and **6b** were verified as follows. Decarboxylation of **6a** and **6b** (LiCl, H_2O–Me_2SO, 170 °C) afforded the known 4-phenylpyridine (Aldrich Chemical Co.) and 3-methyl-4-phenylpyridine: Inoue, H.; Thyagarajan, G.; May, E. L. *J. Heterocycl. Chem.* **1975**, *12*, 709. Abramovitch, R. A.; Saha, M. *Can. J. Chem.* **1966**, *44*, 1765, respectively.
(8) Gould, S. J.; Cane, D. E. *J. Am. Chem. Soc.* **1982**, *104*, 343.
(9) Typified by lavendamycin, see: Doyle, T. W.; Balitz, D. M.; Grulich, R. E.; Nettleton, D. C.; Gould, S. J.; Tann, C.-H.; Moews, A. E. *Tetrahedron Lett.* **1981**, *22*, 4595.

238 (43), 195 (32), 194 (44), 193 (15), 167 (31), 166 (42), 165 (17), 164 (13), 140 (23), 139 (25), 115 (23), 77 (11). Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.18; H, 5.92; N, 3.63.

5-Methyl-4-[2-(benzyloxy)-3,4-dimethoxyphenyl]-2,3,6-tricarbethoxypyridine (6c). A solution of 3,5,6-tricarbethoxy-1,2,4-triazine[5] (126 mg, 0.5 mmol) in $CHCl_3$ (0.5 mL) was treated with 1-pyrrolidino-1-[2-(benzyloxy)-3,4-dimethoxyphenyl]-1-propene (4c; 353 mg, 1.0 mmol, 2.0 equiv) in $CHCl_3$ (0.5 mL) under nitrogen (25 °C), and the resulting dark orange solution was warmed at 45 °C for 3 h. Chromatography of the crude product (SiO_2, 30% ether–pentane eluant) afforded 167 mg (274 mg theoretical, 59%) of pure 6c as a viscous yellow oil: ^1H NMR ($CDCl_3$) δ 7.45–6.76 (7 H, m, aromatic), 5.07 (1 H, d, J = 13 Hz, OCH_2Ph), 4.73 (1 H, d, J = 13 Hz, OCH_2Ph), 4.47 (2 H, q, J = 8 Hz, CH_2), 4.45 (2 H, q, J = 8 Hz, CH_2), 4.15 (2 H, q, J = 8 Hz, CH_2), 3.91 (3 H, s, OCH_3), 3.89 (3 H, s, OCH_3), 2.22 (3 H, s, $ArCH_3$), 1.45 (3 H, t, J = 8 Hz, CH_3), 1.42 (3 H, t, J = 8 Hz, CH_3), 0.98 (3 H, t, J = 8 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.5 (C=O), 165.9 (C=O), 164.4 (C=O), 154.8 (C-4′), 150.3 (C-2), 149.9 (C-6), 147.5 and 142.8 (C-2′/C-4), 137.4 (C-3), 136.5 (C-5), 133.4 (C-8′), 128.4 (C-3′), 128.3 (C-10′), 127.7 (C-11′), 124.6 (C-9′), 124.1 (C-6′), 122.5 (C-1′), 107.5 (C-5′), 75.2 (C-7′), 62.4 (CH_2), 62.0 (CH_2), 61.6 (CH_2), 61.0 (OCH_3), 56.1 (OCH_3), 16.4 ($ArCH_3$), 14.16 (CH_3), 13.99 (CH_3), 13.82 (CH_3); IR (film) ν_{max} 3050, 3000, 2950, 2900, 1725 (C=O), 1595, 1480, 1450, 1370, 1340, 1080, 895, 843, 782, 718 cm^{-1}; mass spectrum, m/e (relative intensity) 551 (M^+, 5), 506 (2), 479 (3), 478 (10), 370 (3), 242 (2), 314 (5), 297 (4), 243 (1), 242 (3), 91 (base), 65 (4). Anal. Calcd for $C_{30}H_{33}NO_9$: C, 65.32; H, 6.03; N, 2.54. Found: C, 64.99; H, 6.28; N, 2.40.

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Registry No. 1, 3930-19-6; 4a, 3433-56-5; 4b, 31889-28-8; 4c, 82521-46-8; 5, 74476-38-3; 6a, 82511-44-2; 6b, 82511-45-3; 6c, 82511-46-4.

Synthesis of Phosphoenolpyruvate and Its Use in Adenosine Triphosphate Cofactor Regeneration[1]

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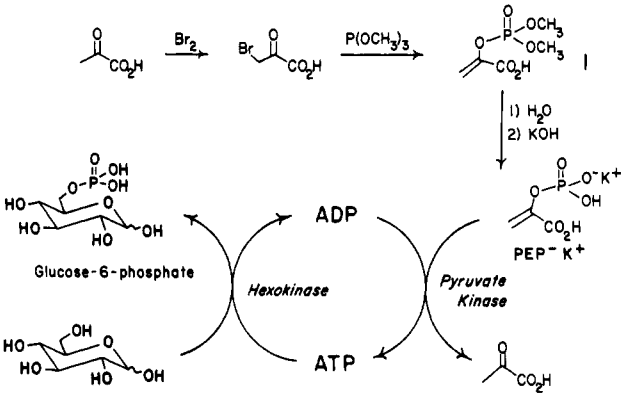
Efficient regeneration of adenosine triphosphate (ATP) in situ from adenosine diphosphate (ADP) is required for many enzyme-catalyzed syntheses. The use of phosphoenolpyruvic acid (PEP) as the ultimate phosphorylating agent for ATP regeneration is an attractive alternative to a procedure reported previously using acetyl phosphate (AcP).[3] Unfortunately, the high cost of PEP has limited its use to analytical-scale reactions.

We report here a convenient synthesis, based on a procedure developed by Clark and Kirby, which can be used to generate PEP in several-mole quantities (Scheme I).[4]

Scheme I. Synthesis of PEP^-K^+ and Use in ATP-Requiring Organic Synthesis



This synthesis has several practical advantages over the previous synthesis. In particular, it accepts crude pyruvic acid as starting material, and it produces PEP directly, in high purity, as an easily handled, stable, crystalline monopotassium salt (PEP^-K^+).[5] We have established the suitability of PEP^-K^+ prepared by this procedure for ATP regeneration using several enzyme-catalyzed syntheses. The preparation of glucose 6-phosphate described here is representative (Scheme I).

ATP regeneration based on PEP^-K^+ and pyruvate kinase has both advantages and disadvantages relative to the scheme based on AcP and acetate kinase.[3] The synthesis and physical properties of PEP^-K^+ are considerably more convenient for laboratory-scale work (several moles) than those of $AcP^{2-}(NH_4^+)_2$,[6] but the cost of the starting materials is higher. PEP is a stronger phosphorylating agent than AcP[7] and can be used more satisfactorily to drive thermodynamically unfavorable reactions.[8] The stability of PEP in solution is much higher than that of AcP.[9] The former can be added in one portion at the beginning of the reaction; the latter must be added continuously. Potassium ion is innocuous as a component of most enzymatic systems (and, in fact, is required for activity of pyruvate kinase[10]); the presence of ammonium ion (from $AcP^{2-}(NH_4^+)_2$) in solution complicates control of the concentration of Mg^{2+}, because $Mg(NH_4)PO_4$ has low solubility in water. Because the K_m of ADP for pyruvate kinase is lower than that for acetate kinase, it is possible to use lower concentrations of A(T,D)P and achieve higher turnover numbers with pyruvate kinase than with acetate kinase.[11]

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(2) NCI predoctoral fellow, CA 09112 CT.

(3) Pollak, A.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1977**, *99*, 2366–2367. Rios-Mercadillo, V. M.; Whitesides, G. M. *Ibid.* **1979**, *101*, 5828–5829.

(4) Clark, V. M.; Kirby, A. J. *Biochem. Prep.* **1966**, *11*, 101–104.

(5) The critical element which renders this preparation suitable for large scale work is the discovery that PEP^-K^+ can be precipitated directly from the crude reaction mixture and used without further manipulation in ATP regeneration.

(6) Lewis, J. M.; Haynie, S. L.; Whitesides, G. M. *J. Org. Chem.* **1979**, *44*, 864–865.

(7) The free energies of hydrolysis in neutral aqueous solution ($\Delta G'_{H_2O}$, kcal/mol) of organic phosphates are representative of their thermodynamic driving force for ATP regeneration. Pertinent values are as follows: PEP, −14.8; creatine phosphate, −10.3; AcP, −10.1; ATP, −7.3; G-6-P, −3.3. Lehninger, A. L. "Biochemistry", 2nd ed., Worth Publishers: New York, 1975; p 398.

(8) We have used PEP^-K^+ to drive phosphorylation of creatine to creatine phosphate in a procedure considerably more straightforward than that described previously with AcP: Shih, Y.-S.; Whitesides, G. M. *J. Org. Chem.* **1977**, *42*, 4165–4166.

(9) The half-lives of PEP and AcP in aqueous solution at 30.5 °C are 98 days (at pH 7.0) and 0.34 days (at pH 7.2), respectively. Benkovic, S. J.; Schray, K. J. *Biochemistry* **1968**, *7*, 4090–4096. DiSabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400–4405.

(10) Lardy, H. A.; Ziegler, J. A. *J. Biol. Chem.* **1945**, *159*, 343–351.

(11) K_m (ADP, pyruvate kinase) = 0.3 mM (McQuate, J. T.; Utter, M. F. *J. Biol. Chem.* **1959**, *234*, 2151–2157). K_m (ADP, acetate kinase) = 1.5 mM (Rose, J. A.; Grumberg-Manago, M.; Korey, S. R.; Ochoa, S. *J. Biol. Chem.* **1954**, *211*, 737–756). The starting concentration of ATP should be ca. 2 K_m.

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