NMR (DMSO-d_6, 361.1 MHz) δ 13.97 (s, OH-1'), 7.37 (s, H-8'), 7.25 (s, H-6), 6.70 (d, J = 1.4 Hz, H-4), 6.55 (s, H-5'), 6.45 (d, J = 1.4 Hz, H-2), 4.84 (d, J = 9.7 Hz, H-1''), 3.93 (s, OCH_3); ^{13}C NMR (DMSO-d_6, 90.8 MHz) δ 184.19 (s, C-9), 179.25 (s, C-9'), 167.11 (s, C-3), 161.97 (s, C-1), 160.93 (s, C-3'), 160.11 (s, C-1'), 157.49 (s, C-4a), 154.97 (s, C-6'), 153.67 (s, C-4a'), 151.14 (s, C-4b'), 150.44 (s, C-8), 144.09 (s, C-7'), 143.27 (s, C-4b), 136.79 (s, C-5), 127.10 (d, C-6), 113.28 (s, C-7), 111.11 (s, C-8a'), 107.57 (s/d, C-8a, C-8'), 106.83 (s, C-2'), 102.87 (s, C-4'), 102.34 (d, C-5'), 102.23 (s, C-8b), 101.31 (s, C-8b'), 97.66 (d, C-2), 93.08 (d, C-4), 81.24 (d, C-5''), 77.98 (d, C-3''), 74.16 (d, C-1''), 71.79 (d, C-2''), 69.45 (d, C-4''), 60.20 (t, C-6''), 56.37 (q, OCH_3); HRMS (positive FAB) m/z (rel int) found [M^+ + H] 695.1248, calcd for $C_{33}H_{27}O_{17}$ [M^+ + H] 695.1251; 695 [M^+ + 1] (100), 677 (9), 631 (8), 617 (19), 603 (19), 589 (7), 575 (13), 571 (7), 569 (13), 561 (24), 559 (14), 557 (17), 545 (14).

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Synthesis and Evaluation of Glucose-ADP Hybrids as Inhibitors of Hexokinase

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Glucose-ADP hybrids, in which carboxamide (**4**) and linear acetylene (**5**) and allene (**6**) functional groups link the two moieties, were designed as potential multisubstrate analogue inhibitors for hexokinase. The diastereomeric aldehydes **15** and **28** were key intermediates in the synthesis of these compounds. Reduction of **15**, amination, and acylation with (diethylphosphono)acetic acid provided the amide phosphonate **20**. Reduction of **15**, three-carbon extension to the propargylic bromide **25**, and Arbuzov displacement gave the acetylenic phosphonate **26**. Addition of ethynylmagnesium bromide to aldehyde **28**, separation of the diastereomers, and Mark rearrangement afforded the allenic phosphonates **31R** and **31S**. Cleavage of the phosphonate esters (trimethylsilyl bromide) and acetal hydrolysis (90% aqueous trifluoroacetic acid) furnished the corresponding deprotected phosphonic acids, which were coupled with adenosine 5'-monophosphate through activation with carbonyl di(imidazole). Inhibition of yeast hexokinase by carboxamide **4** (K_i = 0.2 mM) and acetylene **5** (K_i = 2.5 mM) is competitive with glucose and noncompetitive with ATP; the *R*-allene **6R** (IC_{50} = 1.7 mM) and *S*-allene **6S** (IC_{50} = 10 mM) are also weak inhibitors. It was concluded that these compounds are not functioning as multisubstrate analogues. The β,γ-methylene-γ-methylthio analogue of ATP (**7**) was also synthesized. This compound in combination with glucose, as well as γ-thio-ATP (**9**) in combination with 6-deoxy-6-iodoglucose (**8**), were investigated for potential enzyme-induced, covalent coupling. No evidence for such coupling was observed.

Introduction

Kinases are a ubiquitous and important class of enzymes that transfer the terminal phosphate group from nucleoside triphosphates, usually ATP, to a nucleophilic acceptor. These enzymes play a variety of roles in primary metabolism in the regulation of enzyme activity, in signal transduction, and in the modulation of cellular growth and differentiation.[1] In spite of their importance, no general strategies have been devised for inhibiting kinases. For other broad enzyme classes, such as peptidases and transaminases the synthesis of transition state analogues[2] or suicide inhibitors[3] are effective strategies. These approaches have proven to be elusive for the kinases, however, since an effective mimic has yet to be found for the γ-phosphate moiety in the pentavalent, trigonal bipyramidal geometry that it adopts in the transition state (e.g., **1**) and since no way has been discovered to use such a reaction to trigger a process that could lead to irreversible inactivation of the enzyme.

The ternary nature of the enzyme-substrate complex is a striking feature of the kinase reaction, and it suggests that the design of "multisubstrate analogues"[4] could be an effective approach to the design of inhibitors in which the phosphate acceptor is a small molecule. Indeed, P^1,P^5-di(adenosine-5')pentaphosphate (Ap_5A, **2**), an inhibitor of adenylate kinase, was one of the first multisubstrate analogues to be characterized.[5] Adenylate kinase is strongly inhibited by Ap_5A ($K_i = 2.5 \times 10^{-9}$ M) but not by the homologues with fewer phosphoryl groups in the polyphosphate bridge (Ap_4A: $K_i = 2.4 \times 10^{-5}$ M).[6] The extra phosphate group in Ap_5A is believed to compensate for the

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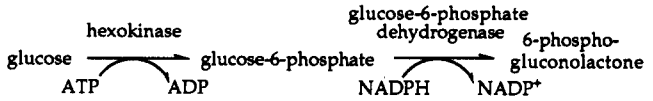
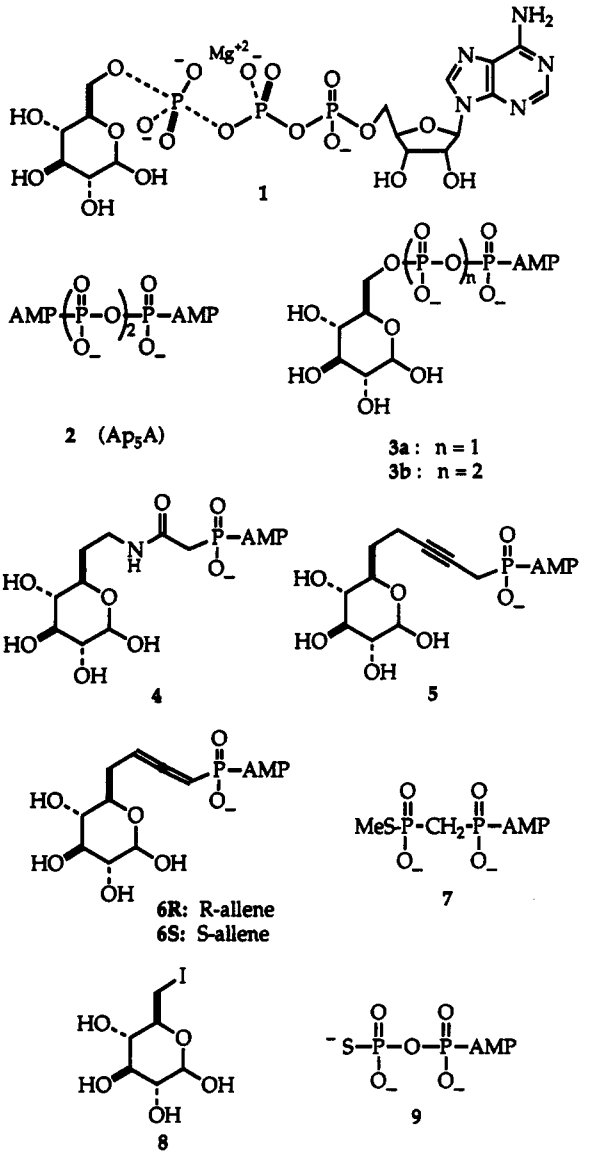
increased bond length of the γ-phosphate in the transition state. Inhibition by Ap_5A is competitive with respect to both substrates, consistent with the proposed bisubstrate analogy.[7]

However, the nucleotide kinases appear to be the only kinases for which this type of bisubstrate analogue leads to potent inhibition.[8] Analogous inhibitors for hexokinase, namely P^1-(glucose-6)-P^4-(adenosine-5′)tetraphosphate (**3a**) and the corresponding triphosphate **3b**, are bound with modest affinity and only compete with ATP.[9] There is evidence that hexokinase, in contrast to adenylate kinase, follows an ordered kinetic mechanism for substrate association and product dissociation and that the binding site for ATP does not exist until the association of glucose induces a protein conformational change.[10] It is thus possible that higher affinity binding for the multisubstrate analogues **3** is blocked for kinetic (as opposed to thermodynamic) reasons. It is also possible that the simple connection of the terminal phosphate of ATP (or its homologue) and glucose via an ester linkage does not allow the ADP unit and the sugar to adopt their optimal positions in the hexokinase binding site.

There are several instances in which multisubstrate analogues have been assembled in the enzyme active site, by covalent bonding of two substrates or substrate analogues to each other. For example, the alkylation of coenzyme-A by bromoacetylcarnitine, which takes place in the ternary complex with carnitine acetyl transferase, leads to an exceedingly potent inhibitor of this enzyme.[11] Cr(III)ATP acts in a similar manner in the presence of glucose and hexokinase: while transfer of the γ-phosphate to the glucose 6-hydroxyl group occurs normally, the covalent attachment of the β- and γ-phosphates to the chromium ion links the two products permanently and results in the formation of a potent multisubstrate analogue (in this instance, "multiproduct" analogue).[12] Such an approach is particularly attractive for the inhibition of protein kinases, for which the prior assembly of multisubstrate analogues may be impractical. However, inhibitors that are based on ATP alone may prove to be indiscriminate inhibitors of all kinases.

Hexokinase catalyzes transfer of the γ-phosphate from ATP to glucose to give glucose-6-phosphate and ADP (eq 1).[12,13] Although there is strong evidence that the transition states for such phosphorylations involve considerable dissociative character,[14] the fact that these transfers invariably occur with inversion of configuration at phosphorus indicates a colinear relationship between incoming and leaving groups.[15] Magnesium(II) is essential for catalysis, coordinating in a bidentate fashion to the β- and γ-phosphates of ATP.[16] Yeast hexokinase has been studied extensively by X-ray crystallography[17] which, together with small-angle X-ray scattering analysis[18] and UV and fluorescence difference spectroscopy,[19] has provided convincing evidence that the enzyme undergoes a significant conformational change upon glucose binding. This induced fit is hypothesized to provide the substrate specificity that prevents hexokinase from acting as an ATPase in the presence of 55 M water.[20]





(7) The crystal structure of the Ap_5A complex of adenylate kinase has been reported by: Egner, U.; Tomasselli, A. G.; Schulz, G. E. *J. Mol. Biol.* **1987**, *195*, 649–658. Interestingly, the authors suggest that one of the adenosine moieties of the inhibitor does not occupy the substrate site of the enzyme.

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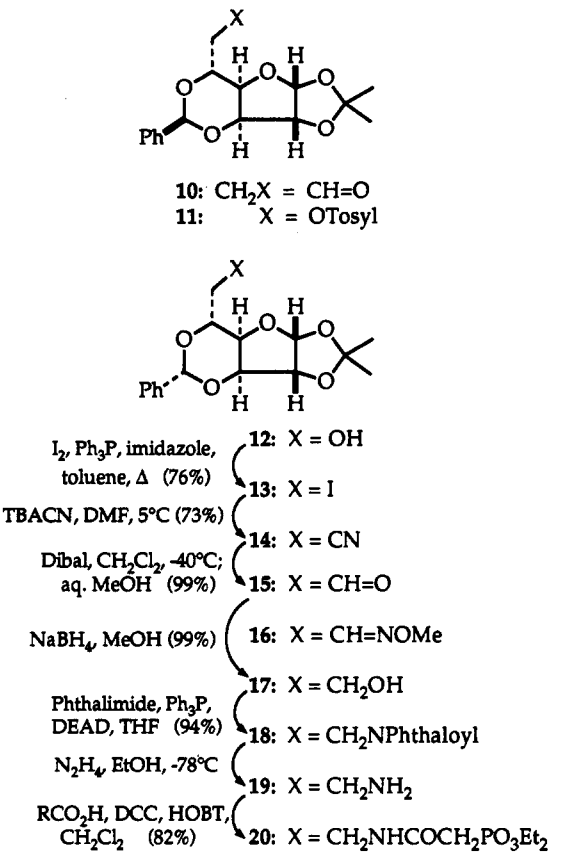
Design

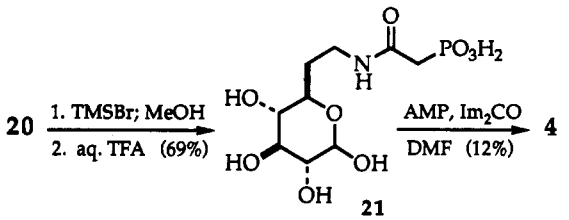
In order to design better multisubstrate analogue inhibitors of hexokinase, we attempted to devise linkers that could position an ADP moiety and glucose mimics in the relative orientation that they adopt in the transition state 1. In designing these linkers, we focused on possible geometric rather than electronic similarities to the trigonal bipyramidal phosphate 1,[21] in particular the linearity of the unit.[22] In carboxamide 4, while the amide $CH_2CONHCH_2$ is not strictly a linear moiety, it does incorporate a polar functional group that could conceivably coordinate the magnesium cation that binds to the β- and γ-phosphates of the ATP substrate. In the acetylene 5, the pentavalent phosphate of 1 is replaced with the four-carbon unit, $CH_2C{\equiv}CCH_2$. This unit spans 3.9 Å, somewhat less than twice the 2.2–2.6 Å P–O bond length proposed for the apical substituents of the pentavalent phosphate.[23] The acetylene linker does allow free rotation of the ADP and glucose moieties relative to each other. In the allenes 6, the three-carbon linker CH=C=CH spans 2.5 Å, considerably less than the pentavalent phosphate. Moreover, the rigidity of the allene unit enforces a perpendicular orientation of the ADP and glucose moieties along the axis of the allene. Allene 6 exists in two diastereomeric forms, and it was conceivable that one would have the appropriate configuration to mimic the transition state 1 in a more fixed manner than acetylene 5.

As an alternative to preformed multisubstrate analogues, two schemes were considered for potential enzyme-assembled inhibitors: in one the ATP moiety was to serve as the electrophile, in the other as a nucleophile. In the thiomethyl phosphonate 7, we sought to block cleavage of the pyrophosphate linkage of ATP by replacing the anhydride oxygen with a methylene group and to provide an alternative leaving group for nucleophilic attack by the glucose 6-hydroxyl. In view of the orientation that these groups would adopt in the active site, the alternative displacement would require pseudorotation at the γ-phosphorus in order to permit substitution with retention of configuration. Although such a process does not occur with "normal" enzyme-catalyzed phosphate transfers, it has been observed in at least one instance with a substrate analogue.[24] For active-site assembly in the opposite sense, we investigated the possible alkylation of 6-deoxy-6-iodoglucose (8) with γ-thio-ATP (9). In both instances, the covalent product would be an analogue of the ATP ester 3b. Although that compound is not a potent inhibitor of hexokinase, the self-assembled analogues could conceivably circumvent any barrier to the binding of 3 as a result of an ordered sequence of substrate binding.

Scheme I



Scheme II



Synthesis

The multisubstrate analogues 4–6 were all synthesized via aldehydes 15 (Scheme I) and 28 (Scheme IV). An attempt to homologate aldehyde 10 with (methoxymethylene)triphenylphosphorane[25] was frustrated by the instability of this aldehyde;[26] an alternative route[27] via the nitrile was therefore developed. Tosylate 11 was obtained

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(22) Models of the putative transition state and proposed inhibitors were superimposed in the active site of yeast hexokinase by computer graphics and compared visually. The starting point for this exercise was the model of ATP-hexokinase derived by Shoham, M. and Steitz, T. A. (*J. Mol. Biol.* **1980**, *140*, 1–14) from the Br-AMP complex. From inspection of this model, it is apparent that further conformational changes take place upon ATP binding: the adenosine portion penetrates the solvent accessible surface of the ATP site and the γ-phosphate is 6 Å from the 6-hydroxyl of glucose. Even though the model is crude, it provides a rough estimate as to how ATP binds to hexokinase. The transition-state model was obtained by bringing the ATP and glucose substrates together until a P–O bond length of 2.4 Å for the apical bonds had been achieved.[23] Each multisubstrate analogue was built utilizing the glucose coordinates from the crystal structure and the ATP-coordinates from the model for the ADP-portion. Standard bond lengths and bond angles were utilized in the construction of the amide, acetylene and allene linkages. Each multisubstrate analogue was superimposed onto the transition-state model and evaluated. The acetylene analogue 5 best mimicked the length and geometry of the transition state. The less rigid amide analogue 4 was also able to reach both substrate sites and to attain a proper geometry. The allenes 6 were somewhat shorter and did not provide as good a match of the substrate sites due to the perpendicular arrangement of the allene function.

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(25) See for example: Boren, H. B.; Eklind, K.; Garegg, P. J.; Lindberg, B.; Pilotti, A. *Acta Chem. Scand.* **1972**, *26*, 4143–4146. Eklind, K.; Garegg, P. J.; Lindberg, B.; Pilotti, A. *Acta Chem. Scand.* **1974**, *28*, 260–261.

(26) Ito, T. *Agr. Biol. Chem.* **1975**, *39*, 273–275.

(27) Garegg, P. J.; Hoffman, J.; Lindberg, B.; Samuelsson, B. *Carbohydr. Res.* **1978**, *67*, 263–266.

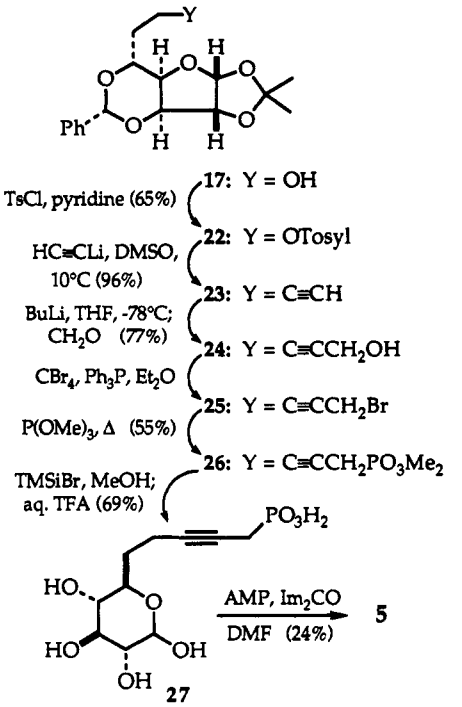
in 96% yield from (*S*)-3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose[28] with tosyl chloride in pyridine.[29] However, direct substitution of this material with tetrabutylammonium cyanide (TBACN; DMF at 5 °C[27]) gave only a 50% yield of the desired product, accompanied by a substantial amount of elimination product. In contrast, iodide **13**,[28] prepared in good yield from the (*R*)-benzylidene alcohol **12** with I_2 and triphenylphosphine,[30] underwent less elimination in the presence of TBACN, furnishing nitrile **14** in 73% isolated yield. Reduction of the nitrile to the aldimine with di(isobutyl)aluminum hydride (Dibal-H)[31] (CH_2Cl_2, −40 °C) and subsequent hydrolysis afforded the 1,7-dialdohepto-1,4-furanose **15** in excellent yield.

Several strategies were investigated for preparation of amine **19** (Scheme I).[32] The most direct route, reduction of nitrile **14**, led to complex product mixtures. Of a variety of reducing agents, $NaBH_4/CoCl_2$[33] was the most effective; however, only a moderate yield of amino diketal **19** was recovered from the strongly acidic workup conditions. Reduction of methoxime **16** with diborane[34] also gave modest yields of the desired amine. Introduction of the amine via the phthalimide **18**[35] proceeded in high yield from alcohol **17**, in turn produced quantitatively by borohydride reduction of aldehyde **15**. Amine **19** was liberated with hydrazine in refluxing EtOH[36] and coupled with (diethylphosphono)acetic acid to give the amide **20** in 65% overall yield from nitrile **14**.

Amide **20** was deprotected in two steps (Scheme II) by ester exchange with an excess of trimethylsilyl bromide[37] (TMSiBr) followed by cleavage of the acetals with 90% aqueous trifluoroacetic acid (TFA).[38] Complete removal of bromide salts proved to be critical for the success of the subsequent coupling reaction with AMP; hence, amide phosphonate **21** was purified by ion-exchange chromatography and reversed-phase HPLC; homogeneous material was obtained in 69% yield from **20**.

Three of the most commonly employed methods for the preparation of nucleotide anhydrides were investigated for the coupling of amide phosphonate **21** and AMP. Reaction of **21** with the commercially available adenosine 5′-monophosphoromorpholidate in pyridine[39] required 3 days at 21 °C and gave rise to several side products, as judged by ^{31}P NMR spectroscopy. While activation of AMP by diphenyl chlorophosphate[40] worked well, the cleanest and most complete reaction was achieved on activation with carbonyl diimidazole.[9b] Although the coupling yield was quantitative, as judged by NMR spectroscopy, considerable losses were encountered on purification by anion exchange chromatography on DEAE Sephadex followed by preparative C_{18} reversed-phase HPLC using triethylammonium bicarbonate (TBK, pH 7.4); the amide **4** was obtained in 12% yield in analytically pure form. The purification procedure was improved considerably during the synthesis of the other analogues **5** and **6**.

Scheme III



Synthesis of the propargylic phosphonate is outlined in Scheme III. Displacement of tosylate from **22** with the dianion of propargyl alcohol or the monoanion of the corresponding *tert*-butyldiphenylsilyl ether proceeded in poor yield; however, addition of **22** to a slurry of lithium acetylide–ethylenediamine complex in DMSO[41] provided the alkylation product **23** in 96% yield on a 5-g scale. Formaldehyde was introduced in gaseous form to the anion of **23** at −78 °C to give alcohol **24** in 68% yield, together with 10% of recovered starting material. Conversion of the alcohol to the bromide and then to the phosphonate via Arbuzov displacement with trimethyl phosphite was accomplished without purification of the intermediate, providing propargylic phosphonate **26** in 55% yield from the alcohol.

Deprotection of phosphonate ester **26** was accomplished in the same manner as described for the amide analogue **20**, affording the free sugar phosphonate **27** in 44% yield. Coupling of **27** to carbonyl diimidazole activated AMP provided acetylene analogue **5** in analytically pure form in 24% yield after anion exchange chromatography. Optimal separation conditions for the final products were achieved on a cellulose resin, eluting with a linear gradient of water/2 N formic acid.[42]

Synthesis of the allenic phosphonates **31** is outlined in Scheme IV. Although the chemistry in Schemes I and III

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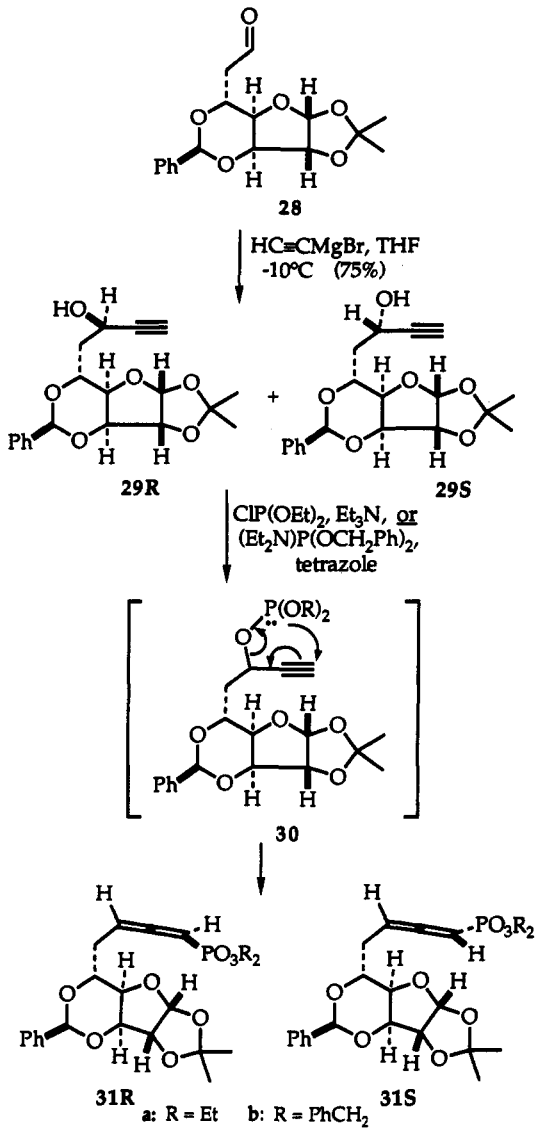
(40) (a) Michelson, A. M. *Biochem. Biophys. Acta* **1964**, *91*, 1–13. (b) Bartlett, P. A.; Eckstein, F. J. *J. Biol. Chem.* **1982**, *257*, 8879–8884.
(41) Smith, B. N.; Beumel, O. F., Jr. *Synthesis* **1974**, 441–442.
(42) Whatman's preswollen DE 52 Cellulose resin gave better resolution in purification of these particular nucleotide anhydride analogs than DEAE Sephadex gel. Chromatography on a LKB prepacked DEAE-5PW FPLC column also served in place of C_{18} reversed-phase HPLC chromatography, which was of importance since we observed a loss of resolution on the preparative C_{18} HPLC column due to deterioration of the resin in TBK buffer. Most importantly, we found that the acidic separation conditions were much more effective than chromatography at neutral pH; we thank Prof. N. Oppenheimer (University of California, San Francisco) for this suggestion.

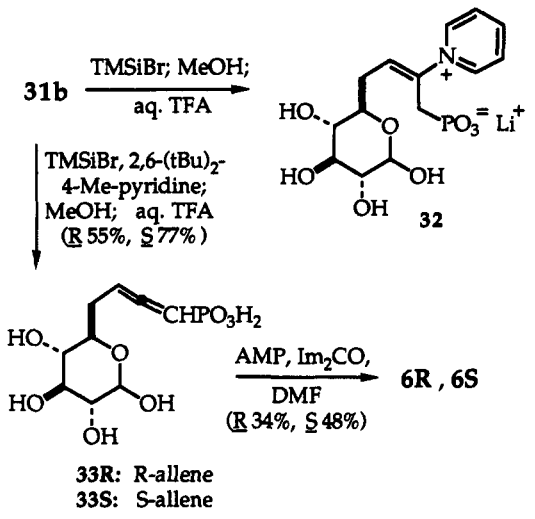
Scheme IV



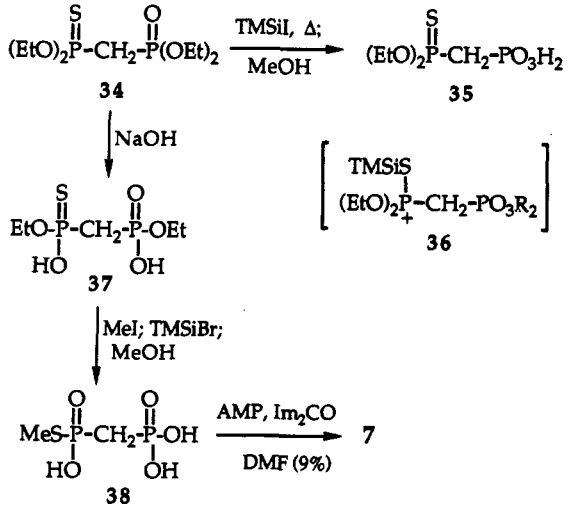
was carried out with the (*R*)-benzylidene acetals, the *S* epimers are depicted in Scheme IV since it was for this series that the diastereomeric propargylic alcohols **29** could be separated readily by chromatography, thereby providing an opportunity to evaluate the individual allene diastereomers. (*S*)-3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose was obtained as the minor diastereomer in the preparation of the *R* acetal **12**[28] and carried on to the aldehyde **28** as described above for the *R* series (**12**–**15**).[43]

The propargylic alcohols **29** were prepared in good yield by the addition of acetylenemagnesium bromide to aldehyde **28**, which was separated into its diastereomers on normal-phase column chromatography. The more polar isomer was converted to the crystalline *p*-nitrobenzoate ester and subjected to single-crystal X-ray analysis, which established the side chain configuration as *S*. Conditions for the Mark rearrangement[44] were developed using the *S* diastereomer of **29** by monitoring the reaction with ^{31}P NMR spectroscopy. After addition of triethylamine and diethyl phosphorochloridite in $CDCl_3$ at 0 °C, acetylenic phosphite **30** was observed at δ 140 ppm, converting to the allenic phosphonate **31aS** at δ 5 ppm within 3 h at room temperature. The product was obtained in good yield (75%) after chromatography.

Scheme V



Scheme VI



The allenic phosphonate functionality proved to be quite susceptible to nuclephilic addition (Scheme V). The allene was destroyed under the conditions required for TMSiBr cleavage of the ethyl esters, for example. The corresponding dibenzyl ester **31bS** was prepared from the propargylic alcohol and *N*,*N*-diethyldibenzylphosphoramidite.[45] Although these ester groups are more labile, TMSiBr and excess pyridine followed by treatment with methanol led to the zwitterionic Michael adduct **32**, as revealed by the ^1H NMR and mass spectra.[46] Even ethyldiisopropylamine gives the corresponding adduct. However, 2,6-di(*tert*-butyl)-4-methylpyridine functioned effectively as a nonnucleophilic proton scavenger in the ester cleavage step, enabling each diastereomer of **31** to be carried through to the fully deprotected allenic phosphonic acids **33R** and **33S** in good yield. Coupling with AMP and purification of the final adducts **6R** and **6S** proceeded as described above for the acetylenic analogue.

(43) Alternatively, the nitrile precursor to aldehyde **28** (the (*S*)-benzylidine isomer of **14**) could be obtained from $AlCl_3$-isomerization of **14**: Harangi, J.; Liptak, A.; Olah, V. A.; Nanasi, P. *Carbohydr. Res.* **1981**, *98*, 165–171. Sznaidman, M.; Cirelli, A. F.; de Lederkremer, R. M. *Ibid.* **1983**, *98*, 326–330.

(44) (a) Mark, V. In *Mechanisms of Molecular Migrations*; Thyagarakan, B. S., Ed.; Wiley-Interscience: New York, 1971; Vol. 2, p 319. (b) Paulsen, H.; Bartsch, W. *Chem. Ber.* **1975**, *108*, 1732.

(45) Perich, J. W.; Johns, R. B. *Tetrahedron Lett.* **1987**, 101–102.

(46) ^1H NMR δ 2.86 (d, 2, J_{HP} = 19.4, CH_2P) and loss of one vinyl proton compared to **31**; MS *m*/*z* = 362 (MH^+).

Although the tetraethyl ester **34** was readily prepared by the procedure of Teaulade and Savignac,[47] its complete deprotection to the tetraacid (in anticipation of S methylation and coupling with AMP) was not straightforward (Scheme VI). Treatment of **34** with an excess of trimethylsilyl iodide (TMSiI)[48] in $CHCl_3$[49] at room temperature or reflux furnished the diester **35**. In the proton-coupled ^{31}P NMR spectrum of the bis(tetrabutylammonium) salt of **35**, the thiophosphonate resonance (δ 96 ppm) was a multiplet, whereas the phosphonate signal (δ 9.5 ppm) appeared as a clean doublet of triplets (J = 17.5, 11.6 Hz), demonstrating that it was the phosphorus–sulfur center that remained esterified. It is clear that the "oxophilicity" of the silyl halide is a crucial factor in the mechanism for ester cleavage by these reagents, such that formation of the (*S*)-silyl intermediate **36** and subsequent dealkylation do not occur.

The synthesis of the monothiol ester of methylenediphosphonate **38** was accomplished in a stepwise fashion. Alkaline hydrolysis of the tetraethyl ester **34** afforded the *O,O'*-diethyl ester **37** in 97% yield after purification on an LH-20 resin. Formation of the disodium salt followed by treatment with methyl iodide[50] afforded the S-methylated product quantitatively. Cleavage of the remaining *O*-alkyl esters was then accomplished with TMSiBr to afford **38**. This material was coupled to AMP, and the adduct **7** was partially purified by anion exchange chromatography. That the diacid end of phosphonate **38** was coupled to AMP was demonstrated by the ^{31}P NMR spectrum of **7**: δ −10 (d, J = 25.4 Hz, α-phosphate), 8 (dd, J = 9.8, 25.4 Hz, β-phosphonate), 36 ppm (d, J = 9.8 Hz, γ-thiophosphonate). The preparation of **7** was not optimized, and homogeneous material (free from starting diphosphonate) was not obtained; this material was therefore tested as a mixture with 15% of **38**.

Evaluation

Hexokinase activity was measured at pH 8 in the coupled assay shown in eq 1, which relies on the reduction of $NADP^+$ to NADPH ($\Delta\epsilon_{340}$ = 6220 M^{-1} cm^{-1}) as the product of the hexokinase reaction is converted to 6-phosphogluconate by glucose-6-phosphate dehydrogenase (G-6-PDH).[51] Complete kinetic evaluations were performed for amide **4** and acetylene **5**, and IC_{50} values were determined for the diastereomeric allenes **6** (Table I, Figure 1). The inhibition constants are much weaker for all of these derivatives than is expected for true multisubstrate analogues. Interestingly, the amide **4** and acetylene **5** are competitive only with glucose, in contrast with the behavior of adenosine-p_3-glucose and adenosine-p_4-glucose, which bind as ATP analogues.[9a] No slow binding of any of the inhibitors was observed over 2 h at 25 °C. Occupancy of the ATP site is presumably blocked by the inability of these compounds to coordinate the magnesium cation that is an important determinant in the binding of ATP. Although the amide linker confers 1 order of magnitude higher affinity on compound **4**, in comparison to the more hydrophobic analogues, the fact that **4** still does not compete with ATP binding suggests that the amide moiety does not serve as a substitute site for magnesium chelation. The 6-fold lower affinity of **6S** as compared to **6R** is presumably related to the manner in which these stereoisomers interact with the active site.

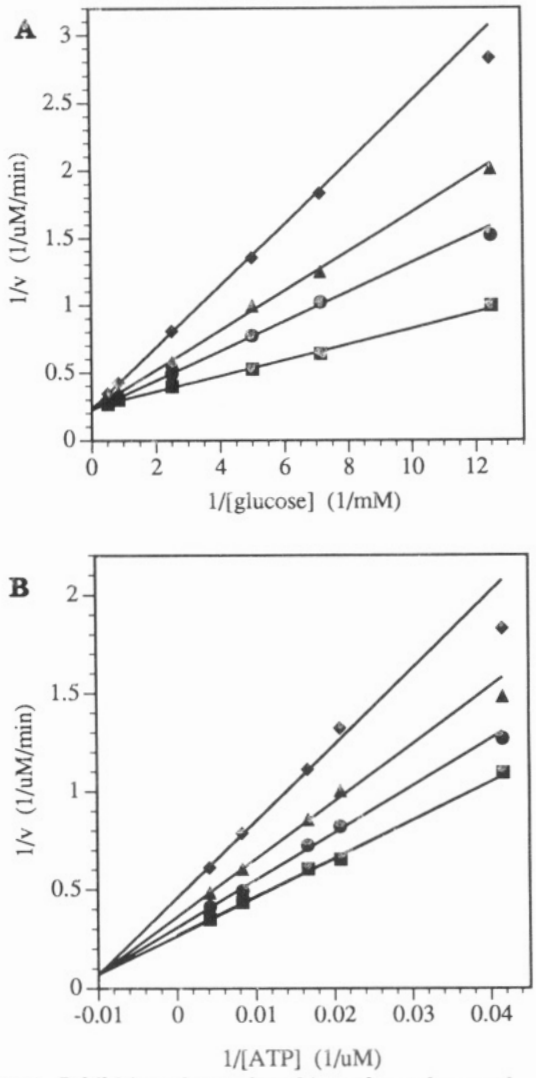


Figure 1. Inhibition of yeast hexokinase by carboxamide **4** ([I] = 0, 138, 276, 552 μM): (A) competition with glucose, [ATP] = 600 μM; (B) competition with ATP, [glucose] = 1.0 mM.

The ATP analogue **7** is also a weak inhibitor of hexokinase (IC_{50} = 3.6 mM), indicating that it does not form the desired multisubstrate analogue in the presence of glucose. Nor does the combination of γ-thio-ATP (**9**) and 6-deoxy-6-iodoglucose (**8**) result in strong inhibition of hexokinase.[52] No slow binding was observed on preincubation of either of these inhibitors with the enzyme over several hours at 25 °C. Had these covalent adducts been formed, inhibition at least comparable to that displayed by adenosine-p_3-glucose would be expected.[9a]

Discussion

A number of factors may contribute to the ineffectiveness of compounds **4**–**6** as multisubstrate analogues. Although they bind to the glucose site and presumably have a geometry compatible with simultaneous occupancy of the ATP-site, the intervening region on the enzyme must not tolerate a hydrophobic linker, in particular one that cannot

(47) Teaulade, M.-P.; Sauvignac, P. *J. Organometal. Chem.* **1986**, *304*, 283–300.

(48) Prepared fresh prior to use according to the method of: Sakurai, H.; Shirahata, A.; Sasaki, K.; Hosomi, A. *Synthesis* **1979**, 740–741.

(49) Blackburn, M.; Ingleson, D. *J. Chem. Soc., Chem. Commun.* **1978**, 870–871.

(50) Kabachnik, M.; Mastryukovka, T. A.; Kurochin, N. I.; Rodionova, N. P.; Popov, E. M. *Zh. Obshch. Khim.* **1956**, *26*, 2278.

(51) Schulze, I. T.; Gazith, J.; Gooding, R. H. *Methods Enzymol.* **1966**, *IX*, 376. *Worthington Enzyme Manual*; Decker, L. A., Ed., Worthington Biochemical Corporation, Freehold, NJ, 1977; p 90.

(52) γ-Thio-ATP is a modest inhibitor of hexokinase with an IC_{50} = 0.6 mM. 6-Deoxy-6-iodoglucopyranose is not an inhibitor of hexokinase, but is a slow substrate for the auxiliary enzyme G-6-PDH.

provide a metal binding site. An additional consideration is the conformational change on the part of the enzyme that forms the ATP binding site after association of glucose. While the crystal structure of the yeast hexokinase–glucose complex shows that the enzyme is in a "closed" form, it is evident that further conformational changes must occur after ATP binds in order for reaction to take place. Although the actual mechanism by which this induced fit is accomplished is not well understood,[53] it is known that binding to the ATP site is influenced by the nature of the ligand in the glucose site.[54] The glucose moiety embedded in the bifunctional derivatives **4–6** may not provide the appropriate trigger to induce binding of the ADP moiety. A similar argument may explain the failure of 6-deoxy-6-iodoglucose to react with γ-thio-ATP in the presence of hexokinase.

Experimental Section

Synthesis.[55] **Note:** IR, ^{1}H NMR, and ^{13}C NMR spectral information for synthetic intermediates is provided in the supplementary material.

***N*-(6,7-Dideoxy-D-*gluco*-heptopyranos-7-yl)-2-[(adenosine-5′-phosphoryl)phosphono]acetamide (4).** Phosphonate dilithium salt **21** (see below) (196 mg, 0.424 mmol) was converted to the dipyridinium salt by passage through a Dowex 50W-X8 cation exchange resin (pyridinium form), elution with water, and lyophilization. The phosphonate was then suspended in dry DMF (5 mL) and tributylamine (303 mL, 1.27 mmol) was added. The mixture was concentrated in vacuo (1 mmHg, 35 °C). Evaporation of three additional volumes of DMF (5 mL) produced a clear oil. Adenosine 5′-monophosphate monohydrate (465 mg, 1.27 mmol) was suspended in dry DMF (6 mL), and tributylamine (606 mL, 2.54 mmol) was added. The solvent was evaporated in vacuo. The residue was rendered anhydrous by repeated evaporations of EtOH and DMF. The bis(tributylammonium) salt of AMP was dissolved in DMF (4 mL), and 1,1′-carbonyldiimidazole (1.03 g, 6.36 mmol) was added. The slurry became clear within 10 min and was allowed to stir at 21 °C for 5 h before it was treated with MeOH (309 μL, 7.63 mmol). After 1 h, the activated AMP was transferred to the bis(tributylammonium) salt of phosphonate **21** with the aid of additional DMF (0.5 mL). The reaction mixture was stirred under N_2 at 21 °C for 35 h before concentration on a rotary evaporator. The thick, pale yellow oil was dissolved in 1:1 MeOH/water (30 mL), and the pH was adjusted to ca. 10 with triethylamine. The product mixture was chromatographed on a DEAE Sephadex A-25-120 (HCO_3 form, 3.0 × 16.5 cm) eluting with a linear gradient of 500 mL each of water and 150 mM TBK, pH 7.0. The 20-mL fractions were monitored at 262 nm. Fractions 44–60 were pooled, lyophilized, and further purified by FPLC (LKB DEAE-5PW, 8 × 75 mm, 10-μm particle size; flow rate 0.7 mL/min; 15-mg injections; 3.5-mL fractions; absorbance monitored at 290 nm) eluting with a gradient of 105 mL each of water and 100 mM TBK, pH 7.3. Three peaks were separated: A (k' = 16.2), B (k' = 18.3), C (k' = 22.0). Some injections contained an impurity eluting before peak A. Peaks A and B (fractions 16–20) were lyophilized to a white powder, and the remaining TBK salts were removed by concentration with 100 mL of MeOH on a rotary evaporator. The chromatography was repeated in order to obtain 37 mg (12%) of the dilithium salt **4** in pure form: ^{1}H NMR (500 MHz, D_2O) δ 8.40 (s, 1), 8.18 (s, 1), 5.96 (d, 1, J = 5.4), 4.99 (d, $^1/_3$, J = 2.5), 4.58 (t, 1, J = 5.3), 4.44 (d, $^2/_3$, J = 8.0), 4.36 (t, 1, J = 5.0), 4.23 (br s, 1), 4.11–4.04 (m, 2), 3.61 (dt, $^1/_3$, J = 2.2, 9.7), 3.46 (t, $^1/_3$, J = 9.6), 3.32 (dd, $^1/_3$, J = 3.8, 9.8), 3.29–3.09 (m, 4), 3.05–2.97 (m, 2), 2.68 (dd, 2, J = 2.3, 21.2), 1.92–1.87 (m, 1), 1.46–1.38 (m, 1); ^{13}C NMR (D_2O) δ 170.07, 152.70, 149.20, 148.71, 142.08, 119.11, 96.62, 92.68, 88.31, 84.74, 84.65, 76.50, 75.30, 75.08, 74.24, 74.07, 73.97, 73.53, 72.42, 70.98, 69.60, 65.86, 39.10, 37.85, 37.16, 36.84, 30.87, 30.69; ^{31}P NMR (D_2O) δ 7.36 (d, J = 25.8), −10.49 (d, J = 25.8); UV λ_{max} 259 nm (ϵ = 14 400 M^{-1} cm^{-1}); MS (FAB) = 657 (MH^+); HRMS calcd for $C_{19}H_{28}N_6O_{15}P_2Li_2$, m/z 657.1485, found 657.1497. Anal. Calcd for $C_{19}H_{28}N_6O_{15}P_2Li_2(H_2O)_4$: C, 31.33; H, 4.98; N, 11.54; P, 8.50. Found: C, 31.75; H, 4.57; N, 11.10; P, 8.50.

5′-Adenylic Acid–6,7,8,9,10-Pentadeoxy-10-phosphono-*gluco*-dec-8-ynopyranose Monoanhydride (5). In an fashion analogous to the preparation of amide **4** described above, the phosphonate dilithium salt **27** (see below) (111 mg, 0.260 mmol) was converted to the bis(tributylammonium) salt via its bis(pyridinium) salt. A solution of imidazole-activated AMP was added, and the mixture was stirred under N_2 at 21 °C for 36 h and concentrated in vacuo. The thick oil was dissolved in 1:1 MeOH/water (30 mL), and the pH of the solution was adjusted to ca. 10 with triethylamine. The mixture was then purified by passage through a DEAE Sephadex A 25-120 resin (HCO_3^- form, 3 × 10 cm) eluting with a linear gradient of 500 mL each of water and 150 mM TBK, pH 7.3. The 25-mL fractions were monitored at 275 nm. Fractions 36–50 were pooled and lyophilized. The product was further purified on a glass-packed FPLC TSK DEAE-5PW Sephadex column (LKB, 10-μm particle size, 8 × 75

Table I. Inhibition of Yeast Hexokinase by Glucose–ADP Analogues[a]

inhibitor	K_i (mM)	inhibition pattern[b] (vs. glucose)	(vs. ATP)
4	0.21 ± 0.01	C	NC
5	2.5 ± 0.3	C	NC
6R	1.7[c]	ND	ND
6S	10[c]	ND	ND
glucose	0.203 ± 0.009 (K_m value)		
ATP	0.061 ± 0.002 (K_m value)		

[a] pH 8.0, 25 °C. [b] C = competitive, NC = noncompetitive, ND = not determined. [c] IC_{50} value.

(53) Cf. Hoggett, J. G.; Kellett, G. L. *Eur. J. Biochem.* **1976**, *68*, 347–353.

(54) See, for example: Viola, R. E.; Raushel, F. M.; Rendina, A. R.; Cleland, W. W. *Biochemistry* **1982**, *21*, 1295–1301.

(55) **General.** Unless otherwise noted, reagents and solvents were purchased from commercial suppliers and used without further purification. Dichloromethane (CH_2Cl_2) and dimethyl sulfoxide (DMSO) were dried by distillation from calcium hydride; toluene was distilled from sodium metal. Tetrahydrofuran (THF), diethyl ether (Et_2O), and benzene were distilled from benzophenone/sodium or potassium ketyl. *N*,*N*-Dimethylformamide (DMF) was dried and distilled under reduced pressure from $MgSO_4$ or $CaSO_4$ and stored over 4-Å molecular sieves. Pyridine was distilled from sodium metal or calcium hydride and stored over potassium pellets. Triethylamine (Et_3N), tributylamine (nBu_3N), trioctylamine, ethyldiisopropylamine, and diethylamine were dried by distillation from calcium hydride and stored over potassium hydroxide pellets. Benzaldehyde dimethyl acetal was distilled prior to use. Zinc chloride was fused three times under high vacuum (1 mmHg) and stored under argon prior to use. Tetrazole was sublimed. Acetylene gas was passed through a −78 °C trap (dry ice/2-propanol), followed by an anhydrous $CaSO_4$ tower. Analytical thin-layer chromatography was performed on precoated plates (0.25 mm, Silica Gel 60 F_{254}, EM Reagents), with visualization by ultraviolet light (254 nm), I_2, 10% H_2SO_4 (aq) spray, anisaldehyde spray (90% of a 5% solution of anisaldehyde in ethanol/5% acetic acid/5% concd H_2SO_4) or by phosphomolybdic acid spray (5% phosphomolybdic acid in methanol). Column chromatography was performed according to the method of Still, Kahn, and Mitra (*J. Org. Chem.* **1978**, *43*, 2923–26) using Silica Gel 60 (230–400 mesh ASTM), EM Reagents. Cation exchange chromatography was performed on AG 50W-X8 (Biorad, 200–400 mesh) or on Dowex 50W-X8 resin. Anion exchange chromatography was carried out on DEAE Sephadex A-40-120 (Sigma), on DE 52 Cellulose (Whatman) resin or on a TSK DEAE-5PW (particle size 10 m, 8.0 × 75 mm) Ultropak FPLC column from LKB. Triethylammonium bicarbonate (TBK) buffer was prepared by addition of carbon dioxide gas to an ice-cooled 1 M solution of Et_3N in water to pH 7.5. All HPLC solvents were filtered and degassed prior to use with Millipore 0.45-mm filters. The boiling points reported for Kugelrohr distillations refer to the oven temperature of the apparatus employed. Melting points are uncorrected. ^{13}C and ^{31}P NMR data were acquired at the frequencies specified using broad-band ^{1}H decoupling. ^{1}H and ^{13}C NMR data obtained in $CDCl_3$ and in CD_3OD are reported in ppm downfield from internal tetramethylsilane as 0.0 ppm (unless otherwise noted); in D_2O solvent, ^{1}H NMR peaks are referred to residual HOD at 4.63 ppm and ^{13}C NMR data are referred to dioxane at 67.4 ppm (Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem.* **1983**, *41*, 27–66). ^{31}P NMR chemical shifts are expressed in ppm on the δ scale (downfield positive) relative to trimethyl phosphate as external standard assigned as 3.086 ppm in $CDCl_3$, 3.49 ppm in CD_3OD and 3.78 ppm in D_2O. ^{1}H NMR data (as well as ^{13}C and ^{31}P NMR data, when appropriate) are tabulated in order: chemical shift (multiplicity, number of protons (or phosphorus atoms), coupling constant in Hertz). Elemental analyses were performed by the Microanalytical Laboratory, and mass spectra were obtained in the Mass Spectrometry Laboratory of the College of Chemistry, University of California, Berkeley.

mm) eluting with a linear gradient of 105 mL each of water and 100 mM TBK, pH 7.3 (20 mg injection size, flow rate 0.7 mL/min, 3.5-mL fractions). Four peaks were separated: A (k' = 7.5), B (k' = 8.5), C (k' = 9.5), and D (k' = 11.0). Peaks B and C (fractions 8–13) were pooled and resubmitted to the same FPLC conditions to obtain pure product, which was converted to 40 mg (24%) of the bis(lithium) salt **5**: ^{1}H NMR (400 MHz, D_2O) δ 8.35 (s, 1), 8.09 (s, 1), 5.91 (d, 1, J = 5.7), 4.93 (d, $^1/_3$, J = 3.8), 4.55 (t, 1, J = 5.3), 4.43 (d, $^2/_3$, J = 8.0), 4.35 (t, 1, J = 4.4), 4.22–4.11 (m, 1), 4.10–4.02 (m, 2), 3.63 (dt, $^1/_3$, J = 2.2, 9.6), 3.44 (t, $^1/_3$, J = 9.5), 3.31–3.23 (m, $^5/_3$), 3.18–2.78 (m, $^5/_3$), 2.49 (d, 2, J = 21.3), 2.16–1.95 (m, 2), 1.87–1.58 (m, 1), 1.39–1.29 (m, 1); ^{13}C NMR δ 152.92, 149.22, 149.03, 142.01, 119.10, 96.62, 92.65, 88.29, 84.80, 84.71, 76.60, 75.34, 75.15, 74.80, 74.21, 74.06, 73.60, 72.49, 71.07, 70.25, 65.86, 30.47, 20.79, 19.40, 15.04; ^{31}P NMR δ 10.76 (br s), −10.62 (br s); UV λ_{max} 259 nm (ϵ = 14 400 M^{-1} cm^{-1}); MS (FAB) m/z 637 (MH^+). Anal. Calcd for $C_{20}H_{27}N_5O_{14}P_2Li_2(H_2O)_{3.5}$: C, 34.30; H, 4.89; N, 10.00; P, 8.84. Found: C, 34.31; H, 4.75; N, 9.99; P, 8.94.

For a representative example of purification using acidic conditions: A sample (40 mg, 70% pure, as judged by ^{31}P NMR spectroscopy) was purified by chromatography on an anion exchange resin (Whatman DE 52 Cellulose, HCO_3^- form, 2.0 × 10 cm; 7 mL fractions) eluting with water–2 N formic acid (500 mL each). Fractions 50–66 were isolated and converted to 22 mg of the bis(lithium) salt **5**, homogeneous as judged by high field NMR analysis.

5′-Adenylic Acid-(*R*)-6,7,8,9-Tetradeoxy-1,2-*O*-isopropylidene-9-phosphono-α-D-*gluco*-nona-7,8-dienopyranose Monoanhydride (6R). As described above for the preparation of amide **4**, the bis(pyridinium) salt of allene **33R** (see below) (95 mg, 0.216 mmol) was treated with imidazole-activated AMP. The reaction mixture was stirred at 21 °C under N_2 for 44 h and then diluted with a 1:1 mixture of MeOH/water (50 mL), and the pH was adjusted to ca. 10 with Et_3N. The crude reaction mixture was applied onto a DEAE Sephadex A-25-120 anion exchange column (HCO_3^- form; 5.0 × 10 cm; 20 mL fractions; absorbance measured at 275 nm) eluting with water–100 mM TBK, pH 7.0 (500 mL each), followed by 100 mM TBK (750 mL), 150 mM TBK (500 mL), and 500 mM TBK (200 mL). Fractions 89–109 were pooled, lyophilized, and subjected to purification on an LKB TSK DEAE-5PW Ultropac column (8.0 × 75 mm; 20-mg injections, flow rate 0.75 mL/min; absorbance monitored at 254 nm) eluting with 0–100 mM TBK, pH 7.0 (0.57%/min). The first major peak that was eluted was the desired product, which was isolated, lyophilized, and converted to the bis(lithium) salt **6R** (51 mg, 34% isolated yield): ^{1}H NMR (400 MHz, D_2O) δ 8.43 (s, 1), 8.24 (s, 1), 5.96 (d, 1, J = 5.3), 5.36–5.31 (m, 1), 5.27–5.17 (m, 1), 4.97 (d, $^1/_3$, J = 3.7), 4.55 (t, 1, J = 5.1), 4.41 (d, $^2/_3$, J = 7.9), 4.35 (t, 1, J = 4.1), 4.23 (s, 1), 4.15–4.03 (m, 2), 3.66 (t, $^1/_3$, J = 9.1), 3.45 (t, $^1/_3$, J = 9.2), 3.34–3.21 (m, $^5/_3$), 3.16–3.01 (m, $^5/_3$), 2.46–2.33 (m, 1), 2.13–1.94 (m, 1); ^{13}C NMR (100.60 MHz) δ 210.93, 210.81, 150.57, 149.00, 145.57, 143.16, 119.15, 96.61, 92.75, 88.75, 87.79, 84.95, 84.86, 76.53, 75.49, 75.24, 75.00, 73.67, 73.53, 73.42, 72.37, 70.98, 65.79, 29.68, 29.61; ^{31}P NMR (161.97 MHz) δ −10.73 (d, J = 24.0), 3.38 (d, J = 24.0); UV λ_{max} 259 nm (ϵ = 15 000 M^{-1} cm^{-1}); MS (FAB^-) m/z 610 $(M - H)^-$, 616 $(M + Li - 2H)^-$. Anal. Calcd for $C_{19}H_{25}N_5O_{14}P_2Li_2(H_2O)_4$: C, 32.82; H, 4.78; N, 10.07; P, 8.91. Found: C, 33.07; H, 4.60; N, 10.16; P, 8.58.

5′-Adenylic Acid-(*S*)-6,7,8,9-Tetradeoxy-1,2-*O*-isopropylidene-9-phosphono-α-D-*gluco*-nona-7,8-dienopyranose Monohydride (6S). According to the procedure for the preparation of amide **4** described above, 182 mg (0.413 mmol) of bis(pyridinium) salt **33S** (see below) was stirred with imidazole-activated AMP for 66 h at 21 °C under N_2. The crude reaction mixture (adjusted to pH ca. 10) was purified by chromatography on a DEAE Sephadex A-25-120 anion exchange resin (5.0 × 10 cm, 20-mL fractions, absorbance monitored at 275 nm) eluting with water–100 mM TBK, pH 7.1 (500 mL each), then 100 mM TBK (500 mL), followed by 150 mM TBK (500 mL). Fractions 85–108 were collected, lyophilized, and resubjected to the same chromatography conditions. Fractions 89–120 were pooled and lyophilized to afford 140 mg (48% crude yield) of the desired product. A sample was further purified by FPLC chromatography on an LKB TSK DEAE-5PW Ultropac column, as described above for the *R* diastereomer, and converted to its bis(lithium) salt (9 mg, 87% pure, as judged by ^{31}P NMR spectroscopy): ^{1}H NMR (400 MHz, D_2O) δ 8.32 (s, 1), 8.07 (s, 1), 5.95 (d, 1, J = 5.9), 5.30–5.28 (m, 1), 5.12–5.07 (m, 1), 4.94 (d, $^1/_3$, J = 3.8), 4.41 (d, $^2/_3$, J = 8.0), 4.35 (t, 1, J = 4.2), 4.20 (t, 1, J = 2.3), 4.00 (dd, 1, J = 2.8, 4.9), 3.64 (dt, $^1/_3$, J = 2.0, 9.0), 3.45 (t, $^1/_3$, J = 9.5), 3.30–3.01 (m, $^1/_3$), 2.40–2.23 (m, 1), 2.18–1.97 (m, 1); ^{13}C NMR (100.60 MHz) δ 210.83, 156.47, 153.75, 149.99, 140.70, 119.50, 96.68, 92.83, 87.69, 84.84, 84.75, 83.92, 76.54, 75.30, 75.18, 75.08, 75.01, 73.54, 73.18, 72.47, 71.30, 65.89, 29.70, 29.65; ^{31}P NMR (161.97 MHz) δ −10.79 (d, J = 24.5, $^1/_3$ P), 2.78 (d, J = 24.5, $^2/_3$ P), 2.72 (d, J = 24.5, $^1/_3$ P); UV λ_{max} 259 nm (ϵ 14 000 M^{-1} cm^{-1}).

Another sample was subjected to chromatography on a DE 52 Cellulose resin (Whatman, HCO_3^- form, 2.7 × 6.5 cm, 10-mL fractions, absorbance monitored at 259 nm) eluting with a gradient of water–2 N formic acid (350 mL each). Fractions 47–65 were collected, lyophilized, and converted to the bis(lithium) salt (14 mg, homogeneous by high field NMR spectroscopy).

γ-Methylthio Adenosine 5′-(β,γ-μ-Methylene)triphosphate (AMPPCH$_2$PSMe) 7. According to the procedure for the preparation of amide **4**, 194 mg (0.941 mmol) of (*S*-methylthio)phosphonomethanephosphonate **38** (see below) was converted to the bis(tributylammonium) salt by treatment with 1.57 mL (6.59 mmol) of tributylamine in DMF. This salt was then stirred with imidazole-activated AMP (1.03 g, 2.82 mmol) under N_2 in DMF (13 mL) for 31 h at 21 °C. The product mixture contained approximately 50% product, as judged by ^{31}P NMR spectroscopy. The crude product (adjusted to pH ca. 10) was purified by chromatography on a Cellulose DE 52 anion exchange resin (HCO_3^- form, 5.0 × 5.5 cm, 10-mL fractions), eluting with a linear gradient of water–100 mM TBK buffer, pH 7.3 (1 L), followed by 100–200 mM TBK (1 L). The cleanest fraction (fractions 86–106) was isolated and passed through a Li^+ cation exchange column to afford 53 mg of an 85:15 ratio of product **7** (9% yield) to starting material **38**: ^{1}H NMR (400 MHz, D_2O) δ 8.27 (s, 1), 8.22 (s, 1), 5.91 (d, 1, J = 5.5), 4.06 (t, 1, J_{obs} = 5.4), 3.74 (t, 1, J_{obs} = 4.4), 4.20 (br s, 1), 4.04 (br s, 2), 2.39 (t, 2, J = 19.0), 1.98 (d, 3, J = 12.6); ^{13}C NMR δ 156.36, 153.63, 149.82, 140.62, 119.37, 87.74, 84.65 (d, J_P = 9.7), 75.17, 71.13, 67.41, 65.80, 35.49 (dd, J_P = 92.5, 129.8), 12.83 (d, J_P = 3.5); ^{31}P NMR δ 35.67 (d, 1, J = 9.8), 7.97 (dd, 1, J = 9.8, 25.4), −10.25 (d, 1, J = 25.4); HRMS (FAB^+) calcd for $C_{12}H_{18}P_3N_5O_{11}SLi_3$ m/z 554.047, found 554.0433.

3,5-*O*-(*R*)-Benzylidene-6-cyano-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (14). Tetrabutylammonium cyanide (6.63 g, 24.7 mmol) was added to an ice-cooled solution of iodide **13**[28] (4.43 g, 9.87 mmol) in 35 mL of DMF. The mixture was kept at 8 °C for 2 h and then partitioned between 250 mL of 1:1 toluene/CH_2Cl_2 and 200 mL of water. The aqueous layer was extracted with two 50-mL portions of 1:1 toluene/CH_2Cl_2, and the combined organic layers were washed with three 100-mL portions of water, dried, and concentrated in vacuo to give 5.29 g of crude product. Chromatography (1:3 EtOAc/hexane) afforded 429 mg of elimination side product and 2.62 g (84%) of cyanide **14** as a colorless, viscous oil: $[\alpha]_D^{24}$ +61° (c 1.4, $CHCl_3$); IR; ^{1}H NMR; ^{13}C NMR; MS (FAB) m/z 318 (MH^+). Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.38; H, 6.12; N, 4.34. Elimination product: ^{1}H NMR.

3,5-*O*-(*R*)-Benzylidene-6-deoxy-1,2-*O*-isopropylidene-7-(methoxyimino)-α-D-*gluco*-hepto-1,4-furanose (16). To a solution of aldehyde **15** (248 mg, 0.774 mmol) (prepared as described below for the preparation of alcohol **17**) in CH_2Cl_2 (4 mL), water (1 mL), and pyridine (0.3 mL, 3.68 mmol) was added methoxyamine hydrochloride (192 mg, 2.30 mmol). The resulting solution was stirred at 21 °C for 40 min, diluted with CH_2Cl_2 (20 mL), washed twice with 30 mL of cold 1 N HCl and with 30 mL of saturated $NaHCO_3$, and worked up to afford 293 mg of crude product which was purified by chromatography (1:3 EtOAc/hexane) to give 216 mg (80%) of oxime **16** a colorless oil: ^{1}H NMR.

3,5-*O*-(*R*)-Benzylidene-6-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*-hepto-1,4-furanose (17). To a magnetically stirred solution of nitrile **14** (5.09 g, 16.04 mmol) in 40 mL of dry CH_2Cl_2 at −40 °C under N_2 was added Dibal-H (24.06 mL of a 1.0 M solution in hexane, 24.06 mmol) over 35 min. The reaction mixture was stirred for an additional 55 min and then quenched at −40 °C with 10 mL of MeOH, followed by 25 mL of water. The cooling bath was removed, and the reaction mixture was stirred at 21 °C until a thick precipitate had formed, which was filtered through Celite. The filter cake was washed with several portions of CH_2Cl_2,

and the combined organic layers were washed with 250 mL each of 10% AcOH and saturated $NaHCO_3$ and worked up. The crude aldehyde **15** (≤16 mmol) was dissolved in 250 mL of MeOH and cooled to 0 °C, and a solution of $NaBH_4$ (6.07 g, 160.4 mmol) in 100 mL of MeOH was added in one portion. After stirring at 21 °C for 20 min, the reaction mixture was diluted with acetone (60 mL) and stirred for 10 min before concentration in vacuo to a thick slurry. The residue was partitioned between 250 mL each of $CHCl_3$ and brine and worked up to give 5.06 g (98% crude yield) of alcohol **17** as a yellow-white solid, homogeneous by ^1H NMR spectroscopy. An analytical sample was prepared by recrystallization from ether–hexane to give white, fluffy crystals: mp 75–76 °C; $[\alpha]_D^{22}$ +76.7° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR; MS (FAB) *m/z* 323 (MH^+). Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.01; H, 6.87.

3,5-*O*-(*R*)-Benzylidene-6,7-dideoxy-1,2-*O*-isopropylidene-7-phthalimido-α-D-*gluco*-hepto-1,4-furanose (18). To a slurry of alcohol **17** (1.45 g, 4.50 mmol), phthalimide (0.695 g, 4.72 mmol), and triphenylphosphine (1.24 g, 4.72 mmol) in 5.0 mL of dry THF was rapidly added a solution of 1.11 mL (4.72 mmol) of diethyl azodicarboxylate in 2.5 mL of THF at 21 °C. The solution became clear and was stirred at 21 °C under N_2 for 1.5 h before it was concentrated in vacuo to a thick yellow oil. The crude product was chromatographed (1:5 EtOAc–hexane) to furnish 1.94 g (94.5%) of phthalimide **18** as a white amorphous solid. An analytical sample (289 mg) was prepared by a second chromatography (1:7 EtOAc–hexane) to give 251 mg (87%) of a white amorphous solid: $[\alpha]_D^{22}$ +86° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR; MS (EI) *m/z* 451 (M^+), 160 (100). Anal. Calcd for $C_{25}H_{25}NO_7$: C, 66.51; H, 5.58; N, 3.10. Found: C, 66.62; H, 5.56; N, 3.25.

***N*-[(Diethylphosphono)acetyl]-7-amino-3,5-*O*-(*R*)-benzylidene-6,7-dideoxy-1,2-*O*-isopropylidene-α-D-*gluco*-hepto-1,4-furanose (20).** A solution of phthalimide **18** (1.56 g, 3.44 mmol) in ethanol (14 mL) and 80% hydrazine hydrate (0.67 mL, 17.2 mmol) was heated under reflux, whereupon the solution turned yellow and, after 10 min, solidified. Ethanol (14 mL) was added, the cake of solid material was broken up, and the suspension was heated under reflux for 1.5 h. The slurry was concentrated to a yellow-white cake to which was added 100 mL each of 5% KOH and $CHCl_3$. The layers were separated and the aqueous phase was extracted with $CHCl_3$ (two 50-mL portions). The combined organic layers were dried (K_2CO_3) and concentrated in vacuo to give 1.17 g of crude product amine **19**. To an ice-cooled solution of the amine in dry CH_2Cl_2 was added dicyclohexylcarbodiimide (852 mg, 4.13 mmol), 1-hydroxybenzotriazole hydrate (558 mg, 4.13 mmol), and (diethylphosphono)acetic acid (834 mg, 4.13 mmol). The reaction mixture was allowed to stir at 21 °C under N_2 for 8 h and then filtered through a plug of glass wool and concentrated in vacuo. The residue was redissolved in CH_2Cl_2 and filtered through a 40-μm Millipore filter and concentrated on a rotary evaporator. The procedure was repeated, and the crude product was partitioned between 75 mL each of saturated $NaHCO_3$ and $CHCl_3$ and worked up to furnish 2.26 g of a yellow oil. This material was chromatographed (EtOAc) to give 1.40 g (82%) of amide **20** as a light yellow oil: $[\alpha]_D^{24}$ +55° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 24.00; MS (FAB) *m/z* 500 (MH^+). Anal. Calcd for $C_{23}H_{34}NO_9P$: C, 55.30; H, 6.86; N, 2.80; P, 6.20. Found: C, 54.97; H, 7.02; N, 2.76; P, 6.10.

***N*-(6,7-Dideoxy-D-*gluco*-heptopyranos-7-yl)-2-phosphonoacetamide (21).** Dry pyridine (1.20 mL) was added to distilled TMSiBr (2.40 mL) in dry CH_2Cl_2 (6.00 mL) in an oven-dried, septum-covered test tube under Ar. After centrifugation, the supernatant (2.57 mL, 4.63 mmol) was added to phosphonate ester **20** (463 mg, 0.927 mmol), and the solution was allowed to stir at 21 °C under inert atmosphere. After 2.5 h, the reaction mixture was diluted with dry CH_2Cl_2 (5 mL) and concentrated on a rotary evaporator. The product was redissolved in CH_2Cl_2 and concentrated. The process was repeated twice with MeOH to afford 715 mg of a white amorphous solid. The residue was dissolved in 5 mL of 90% aqueous trifluoroacetic acid. After 15 min at 21 °C, the reaction mixture was concentrated in vacuo (1 mmHg, 35 °C). The residue was dissolved in water (50 mL) and chromatographed on a column (3 × 14 cm) of DEAE Sephadex A 25-120, eluting with a linear gradient of 500 mL each of water and 100 mM TBK, pH 8.0, and then 200 mM TBK, pH 8.0. Fractions of 7 mL were collected and analyzed by TLC. One drop from each fraction was tested for bromide ion by the addition of two drops of concd HNO_3, followed by one drop of $AgNO_3$. Fractions 120–176 were pooled. After lyophilization, the white powder was dissolved in MeOH (200 mL) and concentrated. The remaining bromide salts were removed by HPLC chromatography (Whatman ODS-3 (C_{18}), Magnum-20, 22 × 5000 mm) by elution with 100 mM TBK, pH 7.2, at a flow rate of 12 mL/min. The absorbance was monitored at 212 nm. Three peaks were separated: A (k' = 0.59), B (k' = 1.06), and C (k' = 1.49). Peaks B and C were lyophilized to a white solid, and the remaining TBK was removed by concentration with 100 mL of MeOH on a rotary evaporator. The residual white powder was chromatographed on a Dowex 50W-X8 cation exchange resin (Li^+ form), eluting with water, and the fractions containing product were lyophilized to afford 210 mg (0.644 mmol, 69%) of the deprotected phosphonic acid **21** as a white powder: ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 15.80; MS (FAB) *m/z* 328 (MH^+). Anal. Calcd for $C_9H_{16}NO_9PLi_2(H_2O)_4$: C, 27.08; H, 6.06; N, 3.51; P, 7.76. Found: C, 27.26; H, 5.66; N, 3.45; P, 7.60.

3,5-*O*-(*R*)-Benzylidene-6-deoxy-1,2-*O*-isopropylidene-7-*O*-(4-methylbenzenesulfonyl)-α-D-*gluco*-hepto-1,4-furanose (22). *p*-Toluenesulfonyl chloride (364 mg, 1.91 mmol) was added in one portion to an ice-cooled solution of 362 mg (1.12 mmol) of alcohol **17** in 2 mL of dry pyridine. The reaction mixture was kept at 0 °C for 6 h and then partitioned between 50 mL each of saturated $NaHCO_3$ and ether. The aqueous layer was extracted with three 25-mL portions of ether, and the combined organic layers were worked up to afford 470 mg (88%) of a yellow oil. The crude product was chromatographed (1:5 EtOAc/hexane) to give 11 mg (3%) of the chloride and 382 mg (72%) of a colorless oil, which solidified upon standing and was homogeneous by ^1H NMR. Recrystallization from benzene–hexane gave 256 mg (0.54 mmol, 48%) of the tosylate **22** as colorless crystals: mp 99–100 °C; $[\alpha]_D^{24}$ +69° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR; MS (EI) *m/z* 476 (M^+), 172 (100). Anal. Calcd for $C_{24}H_{28}SO_8$: C, 60.49; H, 5.92; S, 6.73. Found: C, 60.61, H, 5.90; S, 6.94.

3,5-*O*-(*R*)-Benzylidene-6,7,8,9-tetradeoxy-1,2-*O*-isopropylidene-α-D-*gluco*-non-8-yno-1,4-furanose (23). A dry round-bottomed flask was charged with 1.92 g (18.8 mmol) of lithium acetylide–ethylenediamine complex and 9.0 mL of dry DMSO under inert atmosphere. The slurry was stirred for 15 min at 21 °C, then brought to 10 °C, and the tosylate **22** (4.47 g, 9.38 mmol) in 14.0 mL of DMSO was added dropwise over 5 min. After being stirred at 10 °C for 30 min, the reaction mixture was partitioned between water and 50 mL of CH_2Cl_2. The organic layer was washed with 150 mL each of 1 N HCl and saturated $NaHCO_3$ and worked up to afford a yellow oil. The crude product was purified by chromatography (1:5 EtOAc–hexane) to furnish 2.96 g (96%) of the acetylene as a clear, slightly yellow oil: $[\alpha]_D^{24}$ +96° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR; MS (FAB) *m/z* 331 (MH^+). Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68.84; H, 6.65.

3,5-*O*-(*R*)-Benzylidene-6,7,8,9-tetradeoxy-1,2-*O*-isopropylidene-α-D-*gluco*-dec-8-yno-1,4-furanose (24). An oven-dried, three-neck, round-bottomed flask was charged with a solution of acetylene **23** (1.36 g, 4.12 mmol) in dry THF (5.5 mL). *n*-Butyllithium (3.64 mL, 1.70 M solution in hexane) was added at −78 °C over 3 min under inert atmosphere, and the mixture was stirred for 15 min before formaldehyde was added. The formaldehyde gas inlet consisted of a Pasteur pipet connected to a T-tube on a one-neck 50-mL round-bottomed flask in which 1.86 g (61.8 mmol) of dry paraformaldehyde was placed. The paraformaldehyde was cracked using a heat gun and bubbled into the brown acetylide solution at −78 °C with the aid of a stream of N_2 gas. The reaction mixture was quenched after 10 min with saturated NH_4Cl, concentrated, partitioned between $CHCl_3$ (150 mL) and water (200 mL), and worked up. The crude product (1.65 g) was purified by chromatography (1:3 EtOAc/hexane) to afford 199 mg (15%) of recovered starting material **23** and 1.14 g (77%) of the propargylic alcohol **24** as a pale yellow oil: $[\alpha]_D^{24}$ +52° (*c* 0.5, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR (DEPT). Anal. Calcd for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.67; H, 6.81.

Dimethyl [10-(3,5-*O*-(*R*)-Benzylidene-6,7,8,9,10-pentadeoxy-1,2-*O*-isopropylidene-α-D-*gluco*-dec-8-yno-1,4-furanosyl)]phosphonate (26). Triphenylphosphine (266 mg,

1.02 mmol) was added to a magnetically stirred solution of alcohol **24** (183 mg, 0.508 mmol) and CBr_4 (338 mg, 1.02 mmol) in Et_2O (0.90 mL) at 21 °C. A white precipitate formed immediately, and the solution turned yellow. After 15 min, the reaction mixture was filtered through Celite, and the filter cake was washed several times with Et_2O. The filtrate was washed with saturated $NaHCO_3$ (30 mL), dried, and concentrated in vacuo. The remaining triphenylphosphine oxide in the product mixture was removed by filtration through a plug of Florisil (1:4 EtOAc–hexane) to give bromide **25** as a clear oil (198 mg, 92%). A small amount (9%) of the epimeric (*S*)-benzylidene diastereomer was observed in the ^1H NMR spectrum. A mixture of the bromide and freshly distilled trimethyl phosphite (180 μL, 1.53 mmol) was heated at reflux for 2.5 h. Workup by concentration of the crude product mixture in vacuo (1 mmHg, 45 °C), followed by flash chromatography on SiO_2, afforded 126 mg (55%) of the phosphonate **26** as a clear oil: IR; ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 25.9 (and 9% of the (*S*)-benzylidene epimer at δ 26.3). Anal. Calcd for $C_{22}H_{29}PO_8$: C, 58.40; H, 6.46; P, 6.85. Found: C, 58.28; H, 6.42; P, 6.78.

On a larger scale, starting with 1.14 g (3.16 mmol) of propargylic alcohol **24**, 540 mg of product **26** was obtained in a 40% overall yield and the (*S*)-benzylidene isomer was the dominant epimer: ^1H NMR; ^{13}C NMR (DEPT).

(6,7,8,9,10-Pentadeoxy-*gluco*-dec-8-ynopyranos-10-yl)phosphonic Acid (27). Dry pyridine (0.400 mL) was added to distilled TMSiBr (0.800 mL) in distilled CH_2Cl_2 (2.00 mL) in an oven-dried test tube. The supernatant (1.43 mL, 2.57 mmol) was added to phosphonate ester **26** (388 mg, 0.57 mmol), and the solution was allowed to stir at 21 °C. After 2.5 h, the reaction mixture was diluted with dry CH_2Cl_2 (10 mL) and concentrated in vacuo. The product was redissolved in CH_2Cl_2 and concentrated on a rotary evaporator. The process was repeated twice with MeOH. The residue was dissolved in 2.0 mL of 90% aqueous trifluoroacetic acid. After 15 min at 21 °C, the reaction mixture was concentrated in vacuo (1 mmHg, 35 °C). The residue was dissolved in water (50 mL) and chromatographed on a column (3 × 15 cm) of DEAE-Sephadex A 25-120 eluting with a linear gradient of 200 mL each of water and 60 mM TBK, pH 7.0, followed by 60 mM TBK, pH 7.0. Fractions of 25 mL were collected and analyzed by TLC and the $AgNO_3$ test (see procedure for amide analogue **6**). Fractions 49–73 were pooled, lyophilized, and resubjected to another DEAE Sephadex anion exchange column, using the same conditions, to remove the remaining bromide salts. The lyophilized product was converted to the dilithium salt and lyophilized to afford 118 mg (44%) of the deprotected phosphonic acid **27** as a white fluffy solid: ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 18.65; MS (FAB) *m/z* 309 (MH^+). Anal. Calcd for $C_{10}H_{15}PO_8Li(H_2O)$: C, 34.90; H, 5.57; P, 9.00. Found: C, 35.21; H, 5.72; P, 9.17.

Isomerization of 3,5-*O*-(*R*)-Benzylidene-6-cyano-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (14) to 3,5-*O*-(*S*)-Benzylidene-6-cyano-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose. To a solution of nitrile **14** (855 mg, 2.69 mmol) in dry CH_2Cl_2 (5.4 mL) under N_2 at 21 °C was added 1.08 mL of a 0.1 M solution of $AlCl_3$ in dry Et_2O. The clear solution was stirred under inert atmosphere at 21 °C for 30 min and was then diluted with CH_2Cl_2 followed by saturated $NaHCO_3$ (50 mL each). The aqueous layer was back-extracted with two 25-mL portions of CH_2Cl_2, and the combined organic layers were worked up. Chromatography (1:6 ethyl acetate–hexanes) afforded 111 mg (13.0%) of the less polar (*R*)-benzylidene diastereomer and 662 mg (77.4%) of the desired (*S*)-benzylidene diastereomer, as judged by ^1H NMR spectroscopy. After purification, a white solid was obtained which could be recrystallized from MeOH: mp 150–151 °C; $[\alpha]_D^{22}$ +28.9° (*c* 0.5, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR; MS (EI) *m/z* 317 (M^+), 302 (100). Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.04; N, 4.41. Found: C, 64.32; H, 6.03; N, 4.40.

3,5-*O*-(*S*)-Benzylidene-6-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*-1,7-dialdehydohepto-1,4-furanose (28). As described above for the conversion of **15** to the (*R*)-benzylidene acetal of aldehyde **15**, the (*S*)-benzylidene diastereomer of nitrile **14** (1.97 g, 6.21 mmol) was converted to 1.96 g (99% crude yield) of the aldehyde as a white amorphous solid. This material was carried on directly in the next step. An analytical sample was prepared by chromatography (1:5 EtOAc/hexane): $[\alpha]_D^{22}$ +8.6° (*c* 1.3, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR (DEPT); MS (EI) *m/z* 320 (M^+), 319 (3.71), 113 (100). Anal. Calcd for $C_{17}H_{20}O_6$: C, 63.74; H, 6.29. Found: C, 63.66; H, 6.29.

3,5-*O*-(*S*)-Benzylidene-6,8,9-trideoxy-1,2-*O*-isopropylidene-α-D-*glycero*-D-*gluco*-non-8-yno-1,4-furanose (29S) and 3,5-*O*-(*R*)-Benzylidene-6,8,9-trideoxy-1,2-*O*-isopropylidene-α-L-*glycero*-D-*gluco*-non-8-yno-1,4-furanose (29R). Acetylene gas was bubbled for 10 min through dry THF (15 mL) in an oven-dried three-necked flask equipped with a magnetic stir bar. The solution was cooled to −10 °C, and ethylmagnesium bromide in THF (1.95 mL, 3.10 mmol) was added over 10 min at −10 °C. The reaction mixture was stirred for another 10 min before a solution of the (*S*)-benzylidene diastereomer of aldehyde **15** (663 mg, 2.07 mmol) in 2 mL of THF was added over 15 min. The cooling bath was removed, and the pink reaction mixture was allowed to stir at room temperature for an additional 30 min. The flow of acetylene gas was interrupted, and the resulting yellow reaction mixture was diluted with saturated NH_4Cl. The mixture was extracted with CH_2Cl_2 and worked up to afford 714 mg of a white foam which was purified by chromatography (1:5 EtOAc–hexane) to give 191 mg (32%) of the less polar (faster eluting) **29R** diastereomer and 253 mg (42%) of the more polar diastereomer **29S** as white foams. An analytical sample of the less polar diastereomer **29R** was prepared by HPLC chromatography (1:3 EtOAc–hexane) on a normal-phase semipreparative silica gel column (Rainin, 5-μm, 25-cm) at a flow rate of 3 mL/min, monitoring the absorption at 254 nm. Two peaks were separated, A ($k' = 0.92$) and B ($k' = 1.81$), and peak B as the less polar diastereomer **29R** was collected: $[\alpha]_D^{22}$ +36° (*c* 1.4, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR (DEPT); MS (EI) *m/z* 346 (M^+), 113 (100). Anal. Calcd for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40. Found: 65.63; H, 6.53.

For the more polar **29S** diastereomer: $[\alpha]_D^{22}$ +39° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR (DEPT).

3,5-*O*-(*S*)-Benzylidene-6,8,9-trideoxy-1,2-*O*-isopropylidene-7-(4-nitrobenzoyl)-α-D-*glycero*-D-*gluco*-non-8-yno-1,4-furanose. *p*-Nitrobenzoyl chloride (43 mg, 0.230 mmol) was added to a solution of the more polar propargylic alcohol diastereomer **29S** (53 mg, 0.153 mmol) in dry pyridine (0.75 mL), and the solution was allowed to stir at 21 °C under N_2 for 1.5 h. The mixture was partitioned between 5% K_2CO_3 (25 mL) and CH_2Cl_2 (25 mL), the layers were separated, and the organic phase was worked up. The residue was chromatographed (20% EtOAc–hexanes) to afford 48 mg (65% yield) of the ester as yellow crystals, which were recrystallized from EtOAc–hexanes: mp 204–205 °C; $[\alpha]_D^{22}$ −12° (*c* 0.5, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR. Anal. Calcd for $C_{26}H_{25}NO_9$: C, 63.03; H, 5.09; N, 2.83. Found: C, 62.93; H, 5.26; N, 2.82.

Dibenzyl (*R*)-(3,5-*O*-(*S*)-Benzylidene-6,7,8,9-tetradeoxy-1,2-*O*-isopropylidene-α-D-*gluco*-nona-7,8-dien-9-yl)phosphonate (31bR). *N,N'*-diethyldibenzylphosphoramidite[45] (190 mg, 0.599 mmol) was added to a solution of alcohol **29R** (114 mg, 0.329 mmol) and tetrazole (104 mg, 1.48 mmol) in dry CH_2Cl_2 (0.6 mL). The reaction mixture was stirred under N_2 at 21 °C for 3.5 h, diluted with CH_2Cl_2 (25 mL), washed with 5% $NaHCO_3$ (25 mL), and worked up. The crude product was chromatographed to give 172 mg (89%) of allene phosphonate **31bR** as a clear oil, which solidified upon standing: mp 110–111 °C; $[\alpha]_D^{22}$ −61° (*c* 0.7, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR (DEPT); ^{31}P NMR δ 16.49; MS (FAB) *m/z* 591 (MH^+), 613 (M + Na). Anal. Calcd for $C_{33}H_{35}PO_8$: C, 67.11; H, 5.97; P, 5.24. Found: C, 66.78; H, 6.04; P, 5.31.

On a larger scale (323 mg, 0.930 mmol of alcohol **31bR**), 357 mg (65% yield) of product was isolated after purification.

Dibenzyl (*S*)-(3,5-*O*-(*S*)-Benzylidene-6,7,8,9-tetradeoxy-1,2-*O*-isopropylidene-α-D-*gluco*-nona-7,8-dien-9-yl)phosphonate (31bS). As described above for the other diastereomer, alcohol **29S** (377 mg, 1.09 mmol) was converted to 486 mg (75% yield) of allene phosphonate **31bS** after chromatography. The oil solidified upon standing at 5 °C: mp 100–103 °C; $[\alpha]_D^{22}$ +74° (*c* 1, $CHCl_3$); IR; ^1H NMR; ^{13}C NMR (DEPT); ^{31}P NMR δ 16.61. Anal. Calcd for $C_{33}H_{35}PO_8$: C, 67.11; H, 5.97; P, 5.24. Found: C, 66.85; H, 6.14; P, 5.19.

(*R*)-(6,7,8,9-Tetradeoxy-8-(1-pyridinium)-D-*gluco*-non-7-enopyranos-9-yl)phosphonic Acid (32). This compound was prepared by the procedure described above for the preparation of amide **21**, except 1 equiv of TMSiBr was used. The product

was purified by chromatography on a DEAE Sephadex A 25-120 column, eluting with a linear gradient of 200 mL each of water and 50 mM TBK, pH 8.7. Fractions of 7 mL were collected and analyzed by TLC. Fractions 39–49 were pooled, lyophilized, chromatographed on a Dowex 50W-X8 cation exchange resin (Li^+ form) eluting with water, and lyophilized: ^1H NMR; ^{13}C NMR (DEPT); ^{31}P NMR δ 13.4; MS (FAB) m/z 362 (MH^+), 384 (M + Na), 282 (M – pyridine).

(*R*)-(6,7,8,9-Tetradeoxy-D-*gluco*-nona-7,8-dienopyranos-9-yl)phosphonic Acid (33R). A stock solution of 2,6-di-*tert*-butyl-4-methylpyridine (100 mg, 0.487 mmol) and TMSiBr (0.20 mL, 1.44 mmol) in dry CH_2Cl_2 (0.60 mL) was prepared in a dry vial. The clear solution (132 mL, 0.237 mmol) was added to allene **31bR** (70 mg, 0.118 mmol) dissolved in dry CH_2Cl_2 (0.30 mL). After 3 h at 21 °C under N_2, the reaction mixture was diluted with dry CH_2Cl_2 (5 mL) and worked up as described for amide **21**. The residue was dissolved in 90% aqueous trifluoroacetic acid. After 15 min, the reaction mixture was concentrated in vacuo (35 °C (1 mmHg)). The residue was dissolved in water (30 mL) and chromatographed on a column (3 × 6.5 cm) of DEAE Sephadex A 25-120 eluting with a linear gradient of 200 mL each of water and 100 mM TBK, pH 7.1, and then 250 mM TBK, pH 8.0. Fractions of 7 mL were collected and analyzed by TLC. Fractions 57–70 were pooled, lyophilized and chromatographed on a Dowex 50W-X8 cation exchange resin (Li^+ form) eluting with water, and the product fractions were lyophilized to afford 26.8 mg (77%) of a white powder, which gave no precipitate with $AgNO_3$: ^1H NMR; ^{13}C NMR (DEPT); ^{31}P NMR δ 12.73, 12.66; MS (FAB) m/z 295 (MH^+), 289 ($MH - Li + H)^+$; HRMS calcd for $C_9H_{15}O_8PLi$ m/z 289.0660, found 289.0659.

(*S*)-(6,7,8,9-Tetradeoxy-D-*gluco*-nona-7,8-dienopyranos-9-yl)phosphonic Acid (33S). As described above for the other *R* diastereomer, 449 mg (0.760 mmol) of allene **31bS** was converted to 182 mg (54% isolated yield) of allene **33S** as the pyridinium salt after purification by DEAE Sephadex anion chromatography (3.0 × 11 cm; water–100 mM TBK, pH 7.0 (250 mL each), followed by 100 mM TBK, pH 7.0 (500 mL); 7-mL fractions; fractions 45–82 pooled), followed by cation exchange chromatography on a Dowex 50W-X8 resin (pyridinium form); ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 12.61; MS (FAB^+) m/z 289 ($MH - Li + H)^+$, 295 (MH^+).

***O,O,O,O*-Tetraethyl [(Thiophosphono)methyl]phosphonate (34).** The preparation of this compound followed the procedure described by Teaulade and Sauvignac.[47] An analytical sample was prepared by distillation in a Kugelrohr oven (140 °C (1 mmHg)): IR; ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 84.16 (d, J = 7.4), 19.00 (d, J = 7.4). Anal. Calcd for $C_9H_{22}O_5P_2S$: C, 35.53; H, 7.29; S, 10.54. Found: C, 35.95, H, 7.39; S, 10.64.

***O*-Ethyl [(Ethoxythiophosphinyl)methyl]phosphonate (37).** A heterogeneous mixture of compound **34** (1.00 g, 3.29 mmol) and 2 N NaOH (9.90 mL, 19.7 mmol) was stirred at ambient temperature for 5 d. The clear solution was diluted with H_2O and extracted with $CHCl_3$ (2 × 25 mL). The aqueous layer was acidified with 2 N HCl and extracted with $CHCl_3$–2-propanol (3:1; 8 × 25 mL). The combined organic layers (200 mL) were dried (Na_2SO_4) and concentrated in vacuo to afford 849 mg of a clear, light yellow oil which solidified upon standing. The solid was dissolved in MeOH and chromatographed on an LH-20 resin (Pharmacia, 3.0 × 13.5 cm, 15-mL fractions) eluting with MeOH. Fractions 4–9 were combined and concentrated on a rotary evaporator to give 795 mg (97%) of the diacid **37** as a white-yellow solid: ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 79.31 (d, J = 9.4), 19.28 (d, J = 9.4); HRMS (FAB^+) calcd for $C_5H_{15}O_5P_2S$ m/z 249.0115, found 249.0112.

***O*-Ethyl *S*-Methyl [[(Ethoxyphosphinyl)methyl]thio]phosphonate.** To a solution of thiophosphonate **37** (506 mg, 2.04 mmol) in absolute EtOH (1.5 mL) was added sodium ethoxide (1.88 mL, 1.30 M, 2.45 mmol), followed by methyl iodide (381 mL, 6.12 mmol) at 21 °C under N_2. The round-bottomed flask was placed in a preheated oil bath, and the solution was allowed to reflux for 2 h 10 min. The cooled crude reaction mixture was chromatographed on an LH-20 resin (Pharmacia, 1.5 × 22 cm, 6-mL fractions, eluting with MeOH). Fractions 6–14 were pooled and concentrated in vacuo to give 537 mg (100%) of the thiol ester monoacid as a clear, colorless oil: ^1H NMR; ^{13}C NMR; ^{31}P NMR δ 51.26, 15.69; HRMS (FAB^+) calcd for $C_6H_{17}O_5P_2S$ m/z 263.0273, found 263.0261.

***S*-Methyl [(Thiophosphono)methyl]phosphonate (38).** To an ice-cooled solution of the above compound (494 mg, 1.88 mmol) in dry CH_2Cl_2 (7.0 mL) was added 2,6-di-*tert*-butyl-4-methylpyridine (1.93 g, 9.42 mmol), followed by TMSiBr (2.00 mL, 15.1 mmol). The mixture was stirred at 21 °C for 15 h, diluted with CH_2Cl_2 (10.0 mL), and concentrated on a rotary evaporator. MeOH (2 × 20 mL) was added, and the solvent was removed in vacuo. The mixture was dissolved in 30 mL of 1:2 MeOH–H_2O and chromatographed on a Cellulose anion exchange column (Whatman, 5.5 × 5.0 cm, 10-mL fractions), eluting with a gradient of water–100 mM TBK, pH 7.3 (500 mL each), followed by 100–200 mM TBK, pH 7.3 (500 mL each), to furnish 435 mg (>100%) of isolated product: ^1H NMR; ^{31}P NMR (161.97 MHz) δ 37.98 (br), 19.19 (d, J = 12.3).

Enzymology. General. All assays were followed at 340 nm at 25 °C in a 0.5-mL cuvette. The assay buffer was 50 mM HEPES-NaOH, pH 8.0. Data analysis was accomplished with Varian Multiscan, Kinetic Storage, Enhanced Kinetic Calculations software, or ENZFITTER, a nonlinear regression data analysis program for the IBM PC (by R. J. Leatherbarrow, 1987, and distributed by Elsevier-BIOSOFT, Cambridge, United Kingdom). K_i values were determined from fitting the data to Cleland's programs COMPO, UNCOMP, NCOMP and HYPERO.[56]

Hexokinase. A solution of baker's yeast hexokinase (Baker's yeast, Sigma No. C-302, 1.0 mL, 0.8 mg, 412 U) was dialyzed in a negative-pressure dialyzer against 50 mM HEPES buffer, pH 8.0, at 5 °C to a final volume of ca. 1.5 mL, glycerol was added to 10%, and the solution was stored at 0–5 °C. Enzymatic activity was stable for 1–2 months. Molecular weights of 100 000 for the dimer and 50 000 for the monomeric forms were used in calculations; protein concentration was determined spectrophotometrically at 280 nm, $E^{1\%}_{280}$ = 9.47 for P-II.[57] Working solutions of hexokinase were prepared at 1:500 dilution with buffer containing 10% glycerol and kept at 5 °C for approximately 10 h before use, by which time activity had stabilized; the enzymatic activity was then stable for approximately 24–48 h at 5 °C. The final assay concentrations of hexokinase were 0.5–1 nM.

Glucose-6-phosphate Dehydrogenase. Baker's yeast glucose-6-phosphate dehydrogenase (G-6-PDH, Sigma No. G6378, 3.0 mg, 690 U), obtained as a lyophilized powder, was dissolved in 3.00 mL of buffer and stored at 5 °C.

Reagents. All of the reagents were dissolved in 50 mM HEPES buffer, pH 8.0, which had been filtered through a 0.45-μm Millipore filter. Stock solutions of Mg-ATP (6.00 mM, prepared from ATP (Sigma) and an equimolar amount of $Mg(OAc)_2$) and NADP (Sigma, 20.0 mM) were prepared fresh daily.

Enzyme Assays. All assays were performed in 50 mM HEPES, pH 8.0, at 25 °C in the presence of 0.5–1 nM hexokinase, 50 μg/mL G-6-PDH, 2 mM NADP, and 2 mM $Mg(OAc)_2$. Assay mixtures were prepared in 1.5-mL Eppendorf tubes and equilibrated at 25 °C prior to addition of hexokinase. The reaction was initiated by addition of hexokinase, the solution was centrifuged in an Eppendorf microcentrifuge for 2 s, transferred to a cuvette, and the change in absorbance at 340 nm ($\Delta\epsilon$ = 6220 M^{-1} cm^{-1}) was monitored. Assays were followed for ≤15% of the total reaction. The measured K_m values for glucose and ATP were 203 ± 9 μM and 61 ± 2 μM, respectively.[54]

Inhibitor Stock Solutions. Stock solutions of the inhibitors **4**, **5**, **6R**, and **6S** were prepared in water (ca. 5 mg/mL) with 1 equiv of $Mg(OAc)_2$, and the concentrations were determined spectrophotometrically. The allene **6R** used in the inhibition studies was 90% pure, as judged by ^1H NMR spectroscopy. The amide and acetylene analogues were stable in solution for at least 1 month, as determined by reversed-phase HPLC.

Inhibition by Amide 4 and Acetylene 5. Binding of amide **4** in competition with glucose was determined under the following conditions: [ATP] = 600 μM (ca. 10 × K_m), [glucose] = 40–2000 μM, and [**4**] = 0, 138, 276, and 552 μM. The combined data conformed to competitive inhibition, with K_i = 205 ± 8 μM (Figure 1A).[56] Competition against ATP was determined under the following conditions: [glucose] = 1.0 mM (ca. 5 × K_m), [ATP] = 12–600 μM, and [**4**] = 0, 138, 276, and 552 μM. The observed

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inhibition was noncompetitive versus ATP (Figure 1B). No inhibition was observed in experiments in which the glucose concentration was very high (10 mM, ca. 50 × K_m) and the concentrations of ATP and inhibitor were varied from 24–240 μM and 0–276 μM, respectively.

Inhibition by acetylene **5** was determined under similar conditions; competition with glucose, [ATP] = 600 μM, [glucose] = 80–2000 μM, [**5**] = 0, 454, 908, and 1560 μM, competitive inhibition with K_{is} = 2.5 ± 0.3 mM; competition with ATP, [ATP] = 24–600 μM, [glucose] = 1000 μM, [**5**] = 0, 474, 948, and 1896 μM, noncompetitive inhibition with K_{ii} = 1.7 ± 0.2 mM.

During control experiments in the absence of hexokinase, it was shown that neither **4** at 552 μM nor **5** at 1544 μM, the highest concentrations used in the assays, is a substrate of the coupling enzyme G-6-PDH. Similarly, control experiments in the presence of 2.0 mM NADP, 40 mM glucose-6-phosphate, or 1.0 mM $Mg(OAc)_2$ in buffer with **4** at 552 μM or **5** at 1544 μM revealed no inhibition of the coupling enzyme.

Inhibition by Allenes 6R and 6S. The inhibitor concentrations were varied from 0.6–2.4 mM at [glucose] = 200 μM and [ATP] = 1.2 mM. At each inhibitor concentration, the observed rate of reaction was corrected for the background rate of inhibitor oxidation by G-6-PDH, measured in the absence of hexokinase. IC_{50} values of 1.7 (**6R**) and 10 mM (**6S**) were determined from plots of v_o/v_i versus [I].

Preincubation Studies with 4, 5, 6R, and 6S. Hexokinase was incubated at 25 °C with amide **4** (1380 mM), acetylene **5** (5.8 mM), allene **6R** (3.4 mM), or allene **6S** (2.85 mM), and aliquots were diluted 1:5 with the assay mixture after 0, 1, 2, and 3 h ([glucose] = 400 μM, [ATP] = 120 μM). The rate of loss of enzyme activity was compared to controls which contained hexokinase and buffer in the absence of inhibitor; the half-life of hexokinase under these conditions was about 1 h. Hexokinase was somewhat stabilized in the presence of the amide **4** ($t_{1/2}$ = 2 h); no slow-binding inhibitory behavior was observed for any of the compounds.

Inhibition by Thioester 7. The assay was conducted in the presence of 60 μM ATP (K_m) and 2.0 mM glucose (10 K_m) at inhibitor concentrations of 0–4.5 mM. An IC_{50} for **7** of 3.6 mM was determined from a plot of v_o/v_i vs [I]. In a similar experiment, an IC_{50} value of 12 mM was determined for the diphosphonate **38** present as a contaminant in the preparation of **7**. No increase in the inhibitory activity with time was observed on incubation of 11 mM **7** and 5 mM glucose in the presence of hexokinase over a 3-h period.

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Registry No. **4**·2Li, 136839-32-2; **5**·2Li, 136839-33-3; **6***R*·2Li, 136891-70-8; **6***S*·2Li, 136839-29-7; **7**·3Li, 136839-34-4; **12**, 108865-15-2; **13**, 111056-67-8; (*R*)-**14**, 136839-36-6; (*S*)-**14**, 136839-37-7; **15**, 136839-39-9; **16**, 136839-40-2; **17**, 136839-41-3; **18**, 136839-42-4; **19**, 136839-43-5; **20**, 136839-44-6; **21**, 136839-45-7; **22**, 136839-46-8; **23**, 136839-47-9; **24**, 136839-48-0; **25**, 136839-49-1; (*R*)-**26**, 136856-88-7; (*S*)-**26**, 136839-59-3; **27**, 136839-50-4; **28**, 136839-51-5; **29***R*, 136846-43-0; **29***S*, 136839-30-0; **31b***R*, 136839-53-7; **31b***S*, 136839-31-1; **32**, 136839-54-8; **33***R*, 136839-55-9; **33***S*, 136839-52-6; **34**, 97893-01-1; **35**, 136839-56-0; **37**, 136839-57-1; **38**, 136839-58-2; 3,5-*O*-(*S*)-benzylidene-6,8,9-trideoxy-1,2-*O*-isopropylidene-7-(4-nitrobenzoyl)-α-D-*glycero*-D-*gluco*-non-8-yno-1,4-furanose, 136839-35-5; *O*-ethyl *S*-methyl [[(ethoxyphosphinyl)methyl]thio]phosphonate, 136839-38-8; 5′-adenylic acid, 61-19-8; hexokinase, 9001-51-8.

Supplementary Material Available: IR and NMR data for all synthetic intermediates and kinetic plots for enzyme assays (11 pages). Ordering information is given on any current masthead page.

Stereoselective α-Glycosylation of Nitro Sugar Evernitrose: Synthesis of the Terminal AB Unit of Everninomicin Antibiotics

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The stereoselective α-glycosylation of branched-chain nitro sugar evernitrose (**17**, 2,3,6-trideoxy-3-*C*-methyl-4-*O*-methyl-3-nitro-α-L-*arabino*-hexopyranose) is described. 1-*O*-*p*-Nitrobenzoyl derivatives 18β and 18α were prepared as glycosyl donors starting from evernitrose **17** and its methyl glycoside **12**, respectively. Glycosylation of **18** and 4-*O*-benzoyl-2,6-dideoxy-D-*arabino*-hexopyranoside **15** in CH_2Cl_2 in the presence of TMS triflate promoter at −78 °C gave the α-linked disaccharide **19** exclusively. Alkaline treatment of the protected glycoside **19** led to disaccharide **20**. Curacin derivative **7** and 18α were coupled again by using TMS triflate in CH_2Cl_2 at −78 °C to give crystalline α-linked disaccharide **23** exclusively in 73% yield. Hydrogenolytic cleavage of the phenolic benzyl ether completed the synthesis of the terminal AB unit of everninomicins **8**. The structure and stereochemistry of everninonitrose methyl glycoside **8** have been tentatively deduced from the ^1H NMR spectrum and confirmed by single-crystal X-ray analysis. Reduction of **8** with Al/Hg in aqueous ethanol afforded everninosamine methyl glycoside **9**, the terminal AB unit of antibiotic 13-384 component 5 (**5**).

Introduction

The everninomicins[1,2] are produced by *Micromonospora carbonaceae* and belong to the novel class of orthosomycin oligosaccharide antibiotics.[3] They exhibit excellent in vitro and in vivo activities against Gram-positive bacteria and *Neisseria*, including strains resistant to, e.g., β-lactams, tetracyclines, and macrolides.[4] Studies of the structure-activity relationship in the everninomicin group indicated that the antibacterial activity is associated both with the C–D spiroortholactone linkage and the hydroxyl group in the phenolic ester residue.[1]

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