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DESIGNING INHIBITORS OF DEHYDROQUINATE SYNTHASE: STRUCTURAL SIMPLICITY VERSUS INHIBITORY POTENCY

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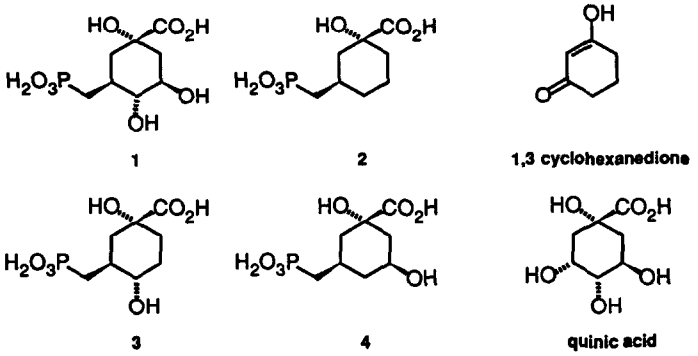
Abstract: Substrate analogues have been synthesized where one or both of the secondary hydroxyl groups have been removed from a carbaphosphonate inhibitor of dehydroquinate synthase. These deoxycarbaphosphonates provide an example of the extent to which substrate analogue synthesis can be shortened and have established the individual and cumulative contributions made by the secondary hydroxyl groups to enzyme inhibition.

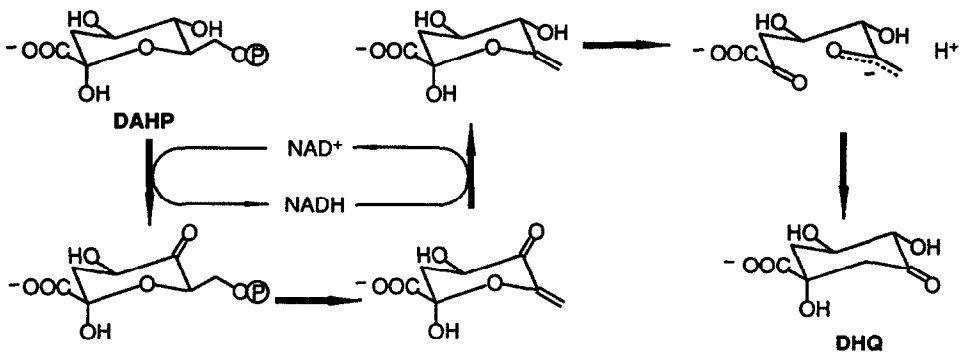
One of the more intriguing steps in the shikimate pathway is the conversion of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) into 3-dehydroquinate (DHQ) catalyzed by the enzyme 3-dehydroquinate synthase. This enzyme has attracted considerable mechanistic interest[1,2,3,4,5,6,7] because of the impressive number of steps (Scheme I) catalyzed by such a comparatively small (MW 40,000 - 44,000)[8] enzyme during each catalyzed turnover of substrate DAHP into product DHQ. Strategies for achieving potent inhibition [2c,d,4b,d,7c,d,e] of DHQ synthase might also serve as paradigms for inhibition of medicinally important enzymes which, like DHQ synthase, share the mechanistic feature of utilizing nicotinamide adenine dinucleotide as a catalyst rather than a cosubstrate.[9] Within the context of aromatic amino acid biosynthesis and the shikimate pathway, appraisal of the biochemical and physiological effects associated with inhibition of DHQ synthase in plant tissue is providing insights into the molecular events which link in vivo inhibition of biosynthetic enzymes with plant death.[7b,e]



Carbaphosphonate 1 is a nanomolar level, slowly reversible inhibitor which amply represents most of the structural features known to be essential to inhibition of 3-dehydroquinate synthase.[4b,7c] Construction of carbaphosphonate 1,[4d,7e] like all known inhibitors[2d,4d,6,7a,d,e,10] of DHQ synthase, requires complicated multistep syntheses. Might simplification of the carbaphosphonate structure lead to substrate analogues which can be more easily synthesized? What is the tradeoff for these analogues between structural simplification and inhibitor potency? To answer these questions, C-4,5 dideoxycarbaphosphonate 2, C-3 monodeoxycarbaphosphonate 3, and

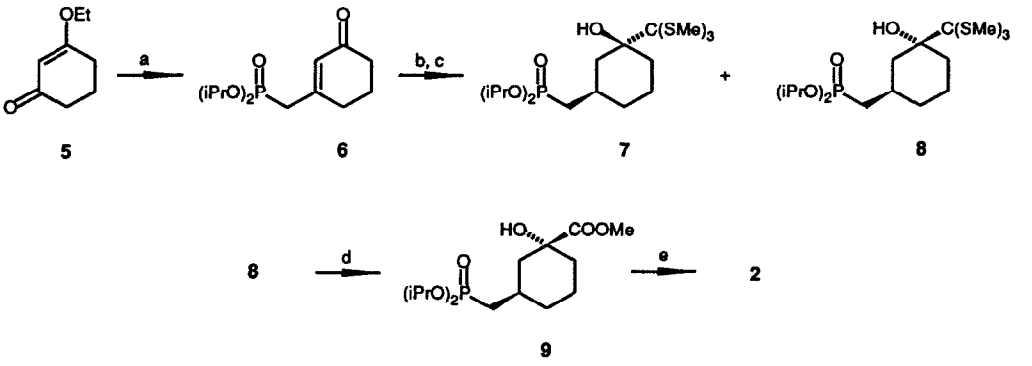
C-4 monodeoxycarbaphosphonate 4 have been synthesized and their inhibition of DHQ synthase appraised. The monodeoxycarbaphosphonates are best viewed as derivatives of quinic acid, while the dideoxycarbaphosphonate is conceptually a derivative of 1,3-cyclohexanedione.

SCHEME I



Assembly (Scheme II) of the dideoxycarbaphosphonate began with addition of 1-lithio-methanephosphonate to the carbonyl of 3-ethoxy-2-cyclohexen-1-one 5. Acidic workup resulted in elimination of the tertiary alcohol and hydrolysis of the enol ether to yield 2-cyclohexen-1-one derivative 6. Catalytic hydrogenation of the conjugated double bond using 10% palladium on carbon afforded a 3-phosphonylmethyl-cyclohexan-1-one which was condensed with the lithium anion of tris(methylthio)methane. This condensation might be cause for concern since racemic mixtures of two diastereomers would be obtained of which only one stereoisomer would be

SCHEME II



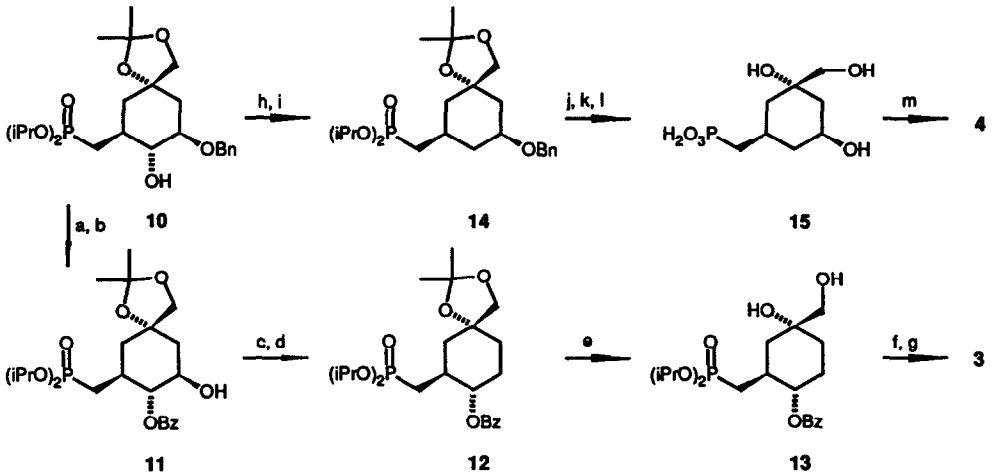
(a) i) $(iPrO)_2P(O)CH_2Li$, THF, -78°C; ii) conc. HCl, THF, RT, 69%; (b) H_2, 10% Pd on C, THF, 100%; (c) $(MeS)_3CLi$, THF, -78°C, (7/8 1:1) 80%; (d) $HgCl_2$, MeOH, RT, 60%; (e) i) TMSBr, Pyr, CH_2Cl_2, RT, ii) H_2O, RT, iii) NaOH, H_2O, RT, iv) Dowex 50 (H^+), 98%.

inhibitory. However, DHQ synthase is insensitive to the stereochemistry of the C-1 asymmetric center as both carbaphosphonate and C-1 epimeric carbaphosphonate are nanomolar-level inhibitors.[7c,e] Therefore, at least two of the four stereoisomers generated by the aforementioned condensation could be DHQ synthase inhibitors. To

simplify synthetic efforts and subsequent measurement of inhibitor kinetic parameters, the two diastereomers (7[11] and 8[12]) which were afforded in nearly equal amounts were separated by silica gel chromatography. A single crystal X-ray analysis of the slower eluting (1:1, hexane/ethyl acetate) diastereomer 8 secured the assignment of the relative stereochemistry of 7 and 8. Stepwise deprotection of the fully protected dideoxycarbaphosphonate 8 began with conversion of the orthothioformate to the methyl ester of 9 after treatment with mercuric chloride and methanol. Hydrolysis of the phosphonate esters was accomplished[13] by reaction of 9 with trimethylsilyl bromide and pyridine in dichloromethane followed by addition of water. After workup with aqueous sodium hydroxide to hydrolyze the methyl ester, purification[14] of the product by anion exchange chromatography provided dideoxycarbaphosphonate 2.[15]

The synthesis of racemic dideoxycarbaphosphonate 2 required 5 steps using inexpensive, achiral starting material and proceeded in 15% overall yield. For comparison, the most efficient synthesis[7e] of carbaphosphonate 1 requires 13 steps using comparatively expensive, chiral quinic acid and is accomplished in 7% overall yield. Simplification of the structure of carbaphosphonate 1 has clearly resulted in a sizable reduction in the number of synthetic steps required to assemble carbocyclic substrate analogues. However, no inhibition of DHQ synthase can be observed even when millimolar concentrations of the dideoxycarbaphosphonate 2 are employed. Therefore, even though dideoxycarbaphosphonate 2 represents one possible extreme in structure simplification, the ease of synthetic assembly has come at the price of a precipitous loss in inhibitor potency.

SCHEME III



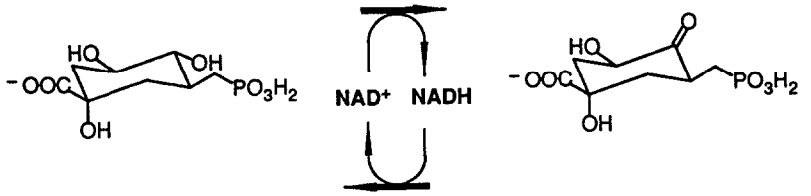
(a) BzCl, Pyr, RT, 79%; (b) H_2, 10% Pd on C, MeOH, 97%; (c) $C_6F_5OC(S)Cl$, N-hydroxysuccinimide, Pyr, benzene, reflux, 78%; (d) n-Bu_3SnH, AIBN, benzene, reflux, 96%; (e) AcOH:H_2O:THF (2:2:1), 70°C, 93%; (f) O_2, Pt, $NaHCO_3$, H_2O:acetone (3:1), 70°C, 86%; (g) i) TMSBr, Pyr, CH_2Cl_2, RT, ii) H_2O, 84%; (h) $C_6F_5OC(S)Cl$, N-hydroxysuccinimide, Pyr, benzene, reflux, 93%; (i) n-Bu_3SnH, AIBN, benzene, reflux, 82%; (j) AcOH:H_2O:THF (2:2:1), 45°C, 94%; (k) H_2, 10% Pd on C, MeOH, 100%; (l) i) TMSBr, Pyr, CH_2Cl_2, RT, ii) H_2O, 95%; (m) O_2, Pt, $NaHCO_3$, H_2O, RT, 57%.

To ascertain whether enzyme inhibition might be retained at an intermediate level of structure simplification, C-3 monodeoxycarbaphosphonate 3 and C-4 monodeoxycarbaphosphonate 4 were synthesized.

A recently elaborated synthesis[7e] of carbaphosphonate **1** from quinic acid proceeded through intermediate **10**. Prepared in seven steps and 30% overall yield from quinic acid, intermediate **10** was ideally suited for selective manipulation of either the C-3 or C-4 hydroxyl groups. Synthesis (Scheme III) of the C-3 deoxycarbaphosphonate **3** began with benzoylation of intermediate **10** followed by hydrogenolysis to yield alcohol **11**. Subsequent Barton-McCombie deoxygenation[16] was complicated by sluggish functionalization of the C-3 hydroxyl group. Formation of the C-3 thionocarbonate ultimately required treating **11** with pentafluorophenyl chlorothionoformate, an equivalent of pyridine, and catalytic N-hydroxysuccinimide in refluxing benzene. The thionocarbonate was purified and then deoxygenated to **12**[17] with tributyltin hydride and AIBN in degassed, refluxing benzene under argon. Removal of the acetonide under mildly, acidic conditions provided diol **13**. The primary alcohol of **13** was subsequently oxidized[18] to a carboxylate with platinum black and molecular oxygen in a heated solution of acetone and water containing sodium bicarbonate. These basic oxidation conditions also resulted in complete debenzoylation and partial hydrolysis of the phosphonate esters to yield a mixture of monoester and diester. This mixture was converted into the desired C-3 deoxycarbaphosphonate **3**[19] by treatment with trimethylsilyl bromide[13] followed by hydrolysis and purification[14] using anion exchange chromatography.

Synthesis (Scheme III) of the C-4 deoxycarbaphosphonate **4** from intermediate **10** closely resembled the synthesis of the C-3 deoxycarbaphosphonate **3**. The free secondary alcohol at C-5 of intermediate **10** was converted to the thionocarbonate which was purified and then subjected to Barton-McCombie deoxygenation.[16] A minute amount of intermediate **10** contaminated deoxygenated **14** and could not be removed by silica gel radial chromatography. The possibility that contamination of **14** with **10** might result in contamination of product C-4 deoxycarbaphosphonate **4** with carbaphosphonate **1** was cause for concern. Even the presence of a fraction of a percent of the nanomolar-level inhibitor carbaphosphonate **1** could misleadingly result in the observation of micromolar-level, slowly reversible inhibition for C-4 deoxycarbaphosphonate **4**. As a consequence, the product of deoxygenation was carefully purified by reverse phase, semi-preparative HPLC. Deoxygenated **14**[20] after this purification was completely free of contamination by **10** as judged by mass spectrometry. Sequential removal of the acetonide, benzyl ether, and phosphonate esters yielded triol **15**. Selective oxidation at room temperature of the primary hydroxyl group with molecular oxygen and platinum black in water containing sodium bicarbonate afforded C-4 monodeoxycarbaphosphonate **4**[21] after purification[14] by anion exchange chromatography.

SCHEME IV



Determination of the inhibition constant (K_i) for C-3 monodeoxycarbaphosphonate **3** and C-4 monodeoxycarbaphosphonate **4** indicated respective values of 2.2×10^{-7}M and 5.4×10^{-7}M. These micromolar-level inhibition constants indicate a tradeoff of approximately 100-fold in inhibitor potency for removal of a single secondary hydroxyl group from the carbocyclic backbone of carbaphosphonate **1**.[4c] It is surprising that the inhibition constants for the two monodeoxycarbaphosphonates are so similar. The nanomolar-level inhibition associated with carbaphosphonate has been attributed to tight interactions between active site residues and the C-4

ketocarbaphosphonate (Scheme IV) formed by oxidation of DHQ synthase-bound carbaphosphonate 1 by bound NAD.[4d] However, removal of the C-4 hydroxyl group is no more deleterious to enzyme inhibition than removal of the C-3 hydroxyl group which plays no direct mechanistic role during conversion of DAHP to DHQ. Therefore, even though monodeoxycarbaphosphonates 3 and 4 are quite respectable inhibitors, the most important dividend to accrue from their synthesis may follow from the questions these inhibitors raise about the underlying basis for potent, slowly reversible inhibition of DHQ synthase.

Acknowledgement. Research was funded by a grant from the National Institutes of Health.

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11. (a) Unless stated otherwise, ^{1}H NMR spectra were recorded at 200 MHz or 300 MHz. Chemical shifts for ^{1}H NMR spectra are reported relative to internal tetramethylsilane with $CDCl_3$ as solvent and to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 when D_2O was solvent. ^{13}C NMR spectra were recorded at 50 MHz or 75 MHz and chemical shifts reported relative to $CDCl_3$ (δ = 77.0 ppm) or CH_3CN (δ = 3.69 ppm). FTIR were recorded in wavenumbers (cm^{-1}).

(b) ^{1}H NMR ($CDCl_3$) δ 4.60-4.75 (m, 2 H), 4.40 (b, 1 H), 1.32-2.50 (m, 11 H), 2.20 (s, 9 H), 1.22-1.30 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 82.4, 80.5, 69.5 (J_{POC} = 7 Hz), 69.2 (J_{POC} = 7 Hz), 33.9 (J_{PCCC} = 4 Hz), 32.5, 31.9 (J_{PCCC} = 8 Hz), 29.4 (J_{PC} = 112 Hz), 27.4 (J_{PCC} = 4 Hz), 23.5 (J_{POCC} = 4 Hz), 16.5, 15.5; IR (neat, NaCl) 3348 (br, m), 2978 (s), 2920 (s), 2872 (m), 2236 (w), 1684 (w), 1452 (m), 1386 (s), 1374 (s), 1310 (w), 1248 (m), 1218 (s), 1178 (m), 1142 (m), 1106 (s), 1012 (s), 982 (s), 890 (m), 868 (w), 816 (w), 764 (w), 732 (s), 644 (m); MS, m/e (rel intensity) EI 277 (33), 193 (100), 107 (22). 86 (11), 84 (17); CI 383 ($M+H^+$-MeSH, 100), 277 (12), 107 (7).

12. ^{1}H NMR ($CDCl_3$) δ 4.55-4.70 (m, 2 H), 2.73 (b, 1 H), 1.40-2.17 (m ,10 H), 2.23 (s, 9 H), 1.27 (d, J= 6 Hz, 12 H), 0.8-1.0 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 81.9 (J_{PCCCC} = 2 Hz), 80.2, 68.9 (J_{POC} = 7 Hz), 40.6 (J_{PCCC} = 11 Hz), 33.6 (J_{PC} = 144 Hz), 32.7 (J_{PCCC} = 11 Hz), 32.2, 28.0 (J_{PCC} = 4 Hz), 23.3 (J_{POCC} = 4 Hz), 21.0, 15.4; IR (neat, NaCl) 3340 (br, m), 2978 (s), 2922 (s), 2234 (w), 1680 (w), 1446 (m), 1386 (s), 1374 (s), 1226 (s), 1178 (s), 1142 (m), 1106 (s), 988 (s), 912 (m), 858 (w), 794 (w), 734 (s), 644 (w); MS, m/e (rel intensity) EI 277 (41), 193 (100), 107 (26), 93 (13), 91 (16); CI 383 ($M+H^+$-MeSH, 100); FAB 861 (M_2+H^+), 453 ($M+Na^+$), 383.

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14. See Reference 7e for a representative procedure for the anion exchange chromatography on AG1 X8 used to purify dideoxycarbaphosphonate **2** and monodeoxycarbaphosphonates **3** and **4**.

15. ^{1}NMR (D_2O) δ 2.00-2.05 (m, 1 H), 1.85-1.96 (m, 2 H), 1.56-1.83 (m, 6 H), 1.59 (dd, J= 13, 13 Hz, 1 H), 1.10 (dd, J = 25, 13 Hz, 1 H); ^{13}C NMR (D_2O) δ 183.0, 77.1, 43.0 (J_{PCCC} = 12 Hz), 35.6 (J_{PC} = 134 Hz), 35.2, 34.9 (J_{PCCC} = 10 Hz), 29.8 (J_{PCC} = 4 Hz), 22.7; MS m/e FAB 331 ($M+H^+$+glycerol), 239 ($M+H^+$), 201; HRMS(FAB) calcd for $C_8H_{15}O_6P$ ($M+H^+$) 239.0685, found 239.0691.

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17. ^{1}H NMR ($CDCl_3$) δ 1.27-1.33 (m, 12 H), 1.41 (s, 3 H), 1.45 (s, 3 H), 1.48-1.65 (m, 3 H), 1.81-1.97 (m, 2 H), 2.00-2.14 (m, 2 H), 2.45-2.62 (m, 2 H), 3.75 (d, J = 8 Hz, 1 H), 3.79 (d, J = 8 Hz, 1 H), 4.62-4.77 (m, 3 H), 7.45 (t, J = 7 Hz, 2 H), 7.57 (t, J = 7 Hz, 1 H), 8.05 (d, J = 7 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 23.5 (J_{POCC} = 5 Hz), 26.8, 26.9, 28.8 (J_{PC} = 142 Hz), 33.0 (J_{PC} = 4 Hz), 33.2, 39.8, 69.2 (J_{POC} = 8 Hz), 69.3 (J_{POC} = 8 Hz), 73.4, 76.0 (J_{PCCC} = 17 Hz), 78.4, 108.8, 127.8, 129.0, 129.7, 132.4, 165.3; IR ($CDCl_3$) 3064 (w), 3034 (w), 2982 (s), 2938 (m), 2872 (m), 1718 (s), 1602 (w), 1452 (m), 1372 (s), 1270 (s), 1110 (s), 1008 (s), 987 (s); MS *m/e* (rel intensity) EI 453 (31), 309 (8), 247 (8), 204 (9), 187 (76), 186 (10), 106 (9), 105 (100), 96 (9), 91 (9), 77 (14); CI 469 (100, $M+H^+$), 411 (30). HRMS (CI) calcd for $C_{24}H_{37}O_7P$ ($M+H^+$): 469.2355, found 469.2365.

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19. ^{1}H NMR (D_2O) δ 1.32 (ddd, J = 16, 16, 3 Hz, 1 H), 1.48-1.98 (m, 8 H), 3.35-3.49 (m, 1 H); ^{13}C NMR (D_2O) δ 35.9 (J_{PCCCC} = 9 Hz), 39.4, 40.3 (J_{PC} = 130 Hz), 42.5 (J_{PCC} = 2 Hz), 47.7 (J_{PCCC} = 10 Hz), 81.7 (J_{PCCC} = 7 Hz), 82.1, 190.5; MS *m/e* (relative intensity) FAB 321 (70, $M+3Na^+-2H^+$), 299 (70, $M+2Na^+-H^+$), 277 (46, $M+Na^+$), 245 (40, $M+H^+$), 137 (100, glycerol+$2Na^+-H^+$); HRMS (FAB) calcd for $C_8H_{15}O_7P$ ($M+2Na^+-H^+$): 299.0273, found 299.0269.

20. ^{1}H NMR ($CDCl_3$) δ 7.32-7.23 (m, 5 H), 4.73-4.60 (m, 2 H), 4.57 (d, J = 12 Hz, 1 H), 4.50 (d, J = 12 Hz, 1 H), 3.76-3.65 (m, 1 H), 3.72 (s, 2 H), 2.41-2.26 (m, 1 H), 2.24-1.93 (m, 3 H), 1.83-1.49 (m, 2 H), 1.34 (d, J = 6 Hz, 12 H), 1.30 (s, 6 H), 1.23-0.88 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 138.7, 128.1, 127.2 (2), 109.3, 80.4, 74.3, 74.0, 69.8, 69.4 (J_{POC} = 7 Hz), 42.4 (J_{PCCC} = 9 Hz), 41.2, 39.2 (J_{PCCC} = 12 Hz), 33.4 (J_{PC} = 141 Hz), 26.8, 26.4, 23.5 (J_{POCC} = 4 Hz); IR (neat, NaCl) 3446 (br, m), 2980 (s), 2934 (s), 2866 (m), 2232 (w), 1496 (w), 1456 (m), 1370 (s), 1246 (s), 1214 (s), 1142 (m), 1104 (s), 1072 (s), 1008 (s), 982 (s), 926 (w), 886 (s), 808 (m), 734 (s), 698 (m); MS, m/e (rel intensity) EI 439 (14), 277 (13), 187 (18), 91 (100); CI 455 ($M+H^+$, 15), 398 (23), 397 (100); HRMS (CI) calcd for $C_{24}H_{39}O_6P$: 455.2563, found 455.2559.

21. ^{1}H NMR (500 MHz, D_2O) δ 3.88-3.94 (m, 1 H), 2.24-2.27 (m, 1 H), 1.95-2.10 (m, 1 H), 1.85-1.95 (m, 1H), 1.78-1.81 (m, 1 H), 1.65 (dd, J = 12, 12 Hz, 1 H), 1.58 (dd, J = 17, 7 Hz, 2 H), 1.46 (dd, J = 13, 13 Hz, 1 H), 1.06 (dd, J = 24, 12 Hz, 1 H); ^{13}C NMR (D_2O) δ 182.0, 78.0, 68.8, 43.8 (J_{PCCC} = 10 Hz), 43.5, 42.8 (J_{PCCC} = 12 Hz), 35.7 (J_{PC} = 134 Hz), 29.4 (J_{PCC} = 4 Hz); HRMS (FAB) calcd for $C_8H_{15}O_7P$: 255.0634, found 255.0635.