Synthesis of Farnesol Analogues Containing Triazoles in Place of Isoprenes through 'Click Chemistry'

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Abstract: A solid-phase three-component Huisgen reaction has been used to generate polar farnesol and farnesyl diphosphate analogues. The Cu(I)-catalyzed 1,3-cycloadditions of various azides with solid supported (*E*)-3-methylhept-2-en-6-yn-1-ol provided only the 1,4-disubstituted 1,2,3-triazole regioisomers. The organic azides were generated in situ to minimize handling of potentially explosive azides. We have employed this powerful 'click chemistry' to make farnesol analogues where both β- and γ-isoprenes were replaced by triazole and substituted aromatic rings, respectively.

Key words: farnesol, farnesyldiphosphate, cancer, cycloaddition, triazoles, solid-phase synthesis, click chemistry

Farnesyl diphosphate (**1**, FPP) analogues have been used to study the mechanism of protein farnesyltransferase (FTase) and to examine the effect of analogue structure on prenylated protein function.[1–6] FTase catalyzes transfer of the isoprenoid to the cysteine residue of proteins with C-terminal CAAX motifs, including the oncoprotein Ras.[7,8] Activating mutations in Ras are found in 30% of all human cancers, and Ras prenylation is obligatory for Ras function.[9–11] Ras function can be inhibited by FTase-catalyzed transfer of FPP analogues with reduced hydrophobicity, such as **2a**,**b** and **3**.[12] The FTase-catalyzed transfer of FPP analogues depends on both analogue structure and CAAX sequence.[13] However, there is no correlation between FPP analogue hydrophobicity and FTase-catalyzed isoprenoid transfer to protein. We have previously shown that an aniline or phenoxy group is isosteric with the terminal isoprene of FPP and that analogues with a range of substituents on the aryl group are FTase-transferable substrates.[14] These observations open the door to FTase-transferable FPP analogues that act as prenyl-function inhibitors (PFI) by interfering with a prenylated protein's function.[12]

We have previously used solid-phase organic synthesis (SPOS) to generate a library of FTase-transferrable FPP analogues where the γ-isoprene was replaced by substituted aryl groups[14,15] (Figure 1). SPOS is particularly useful for many synthetic transformations because excess reagents can be used to drive reactions to completion and facilitates the removal of leftover reagents and soluble byproducts.[16] Cu(I)-catalyzed 1,3-cycloaddition of azides and alkynes to form 1,4-triazoles is a robust way of preparing small-molecule combinatorial libraries by SPOS.[17–20] SPOS has been employed to prepare libraries of 1,4-triazoles where either the azide or alkyne is attached to the solid support.[21–30] Utilizing alkyne-functionalized solid supports prevents formation of Cu(I)-catalyzed alkyne homocoupling side products. Recently, Gibbs et al. reported a library of *S*-farnesyl-thiopropionic acid 1,4-triazoles prepared by solution-phase methods to study their inhibitory activity against isoprenylcysteine methyltransferase (ICMT).[31] Herein, we report a concise SPOS of FPP analogues where the β-isoprene and γ-isoprene were replaced by 1,4-triazole and substituted aryl groups, respectively. The substitutions on the aryl rings were chosen to either increase or decrease the hydrophilicity of the analogues (Scheme 1, Table 2).

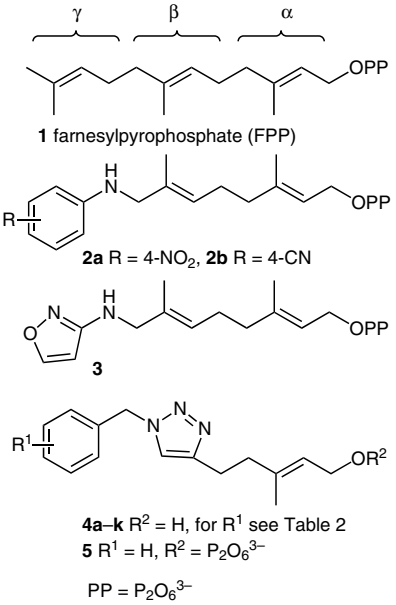

Figure 1 Structure of FPP analogues

Treatment of previously reported[1] alcohol **6** with Ph_3PBr_2 and Hünig's base (DIPEA) gave the pure *E*-isomer of allylic bromide **7**. Propargyl Grignard prepared with a catalytic amount of HgCl was coupled to bromide **7** to give

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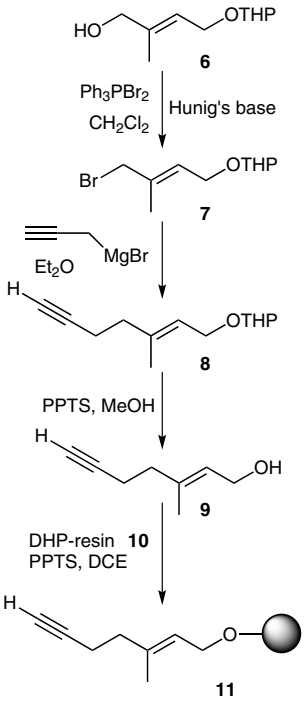
alkyne **8** in 72% yield. Treatment of alkyne **8** with PPTS in MeOH furnished alcohol **9** in quantitative yield.[32] Coupling alcohol **9** to DHP-resin **10** generated the alkyne-functionalized resin **11** with a traceless, acid-labile THP linker.[15]



Scheme 1 Synthesis of resin-linked alkyne **11**

Resin loading of 82% was determined by recovering alcohol **9** from resin **11** treated with PPTS in MeOH and DCE. The key reaction was the 1,4-disubstituted 1,2,3-triazole formation between the resin-linked alkyne and various benzyl azides to allow introduction of diversity in the terminal aryl group.[18,33,34] The required benzyl azides were prepared in situ from commercially available bromides and NaN_3 in DMF. CAUTION! Due to risk of explosion,[35–38] it is crucially important not to use halogenated solvents at this stage and to ensure that all azide ion is removed by thorough washing of the resin before introducing halogenated solvents in any subsequent steps.

Agitating resin **11** with catalytic Cu(I)I and 10 equivalents each of BnBr, NaN_3, and Hünig's base in DMF provided triazole resin **7a**–**k**. A large excess of CuI (>5 equiv) leads to the formation of a side product where iodine is incorporated into the triazole ring **4l**. The structure of (*E*)-5-(1-benzyl-5-iodo-1*H*-1,2,3-triazol-4-yl)-3-methylpent-2-en-1-ol (**4l**) was confirmed by X-ray crystallography (Figure 2).

The in situ generation of Cu(I) from $CuSO_4$ and ascorbic acid in the presence of water inhibited resin swelling and reduced the yield of triazole to almost nil. Triazole yield was also significantly reduced when other protic and nonprotic solvent mixtures drawn from the literature were used. Optimum triazole yields were obtained using Hünig's base and DMF as solvent (Table 1).

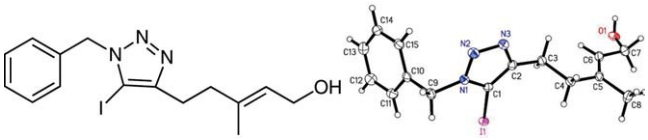


Figure 2 Structure and ellipsoid plot of compound **4l**[41]

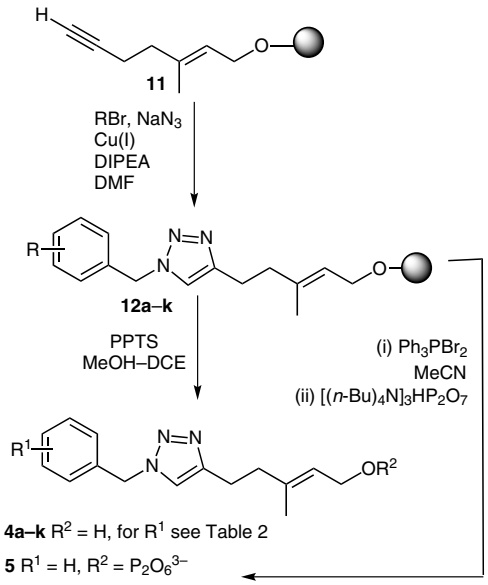
Table 1 Conditions Attempted for Conversion of **7a** into **8a** Using CuI as Catalyst

Entry	Solvent system	Yield (%)[a]
1	DMF–*t*-BuOH (3:1), DIPEA	28
2	DMF–*t*-BuOH–H_2O (3:1:1), DIPEA	0
3	DMF–*t*-BuOH (1:1), DIPEA	32
4	DMF, DIPEA	53
5	DMF, TMED	42

[a] Yields were calculated after cleaving the resin (solvent MeOH–DCE = 1:1).

Heating the resin in MeOH–DCE (1:1) with catalytic PPTS over night provided analogues **4a**–**k** in moderate yield (Scheme 2, Table 2). Prolonged heating of the cleavage reactions resulted in side products formed by 1,3-migration of the hydroxyl group. Exclusive formation of the 1,4-disubstituted 1,2,3-triazole regioisomer was confirmed by ^{1}H-NOE experiments, as expected for the Cu(I)-catalyzed Huisgen reaction.[39]

Triazole diphosphate **5** (19% yield in three steps) was prepared by Ph_3PBr_2 cleavage of THP resin **12a**, followed by trapping the resulting allylic bromide with 10 equivalents of tris(tetra-*n*-butylammonium)hydrogen diphosphate. The crude diphosphate was purified by ion-exchange chromatography followed by RP-HPLC. All compounds



Scheme 2 Synthesis of Triazole-Containing Farnesols **4a**–**k** and diphosphate **5**

in Table 1 were fully characterized by ^{1}H NMR spectroscopy and low- and high-resolution mass spectrometry.[40]

In summary, we have developed a short synthetic route to triazole-containing farnesol analogues and their diphosphates through solid-phase 'click chemistry'. The biochemical characterization of these compounds is ongoing and will be published elsewhere.

Acknowledgment

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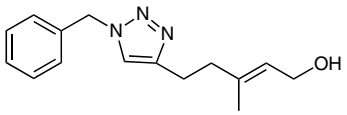
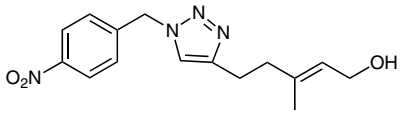
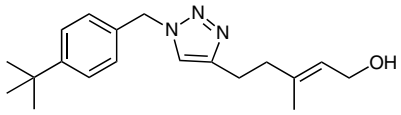
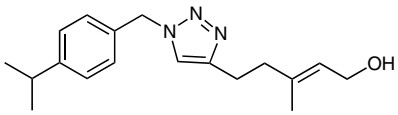
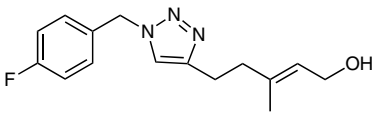
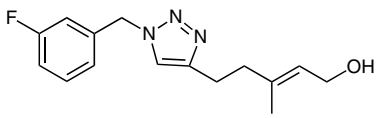
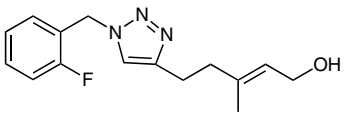
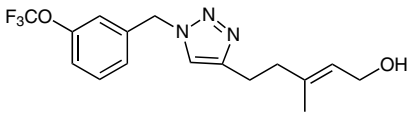
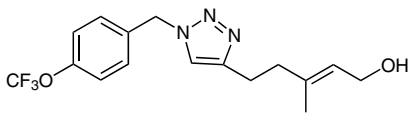
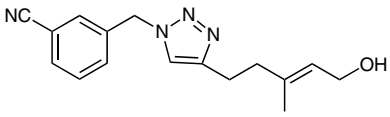
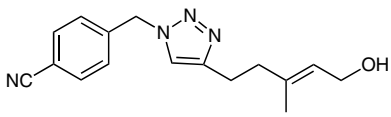
Table 2 1,2,3-Triazole-Containing Farnesol Analogues

Entry	Compound	Structure	Yield (%)[a]	Purity LC–MS (%)[b]
1	**4a**		53	96
2	**4b**		55	89
3	**4c**		43	96
4	**4d**		36	88[c]
5	**4e**		26	94[c]
6	**4f**		57	90
7	**4g**		41	94
8	**4h**		32	98
9	**4i**		28	98
10	**4j**		38	94
11	**4k**		47	98

[a] Yield (%) for **4a**–**k** in two steps from **11**. Dehydrated side products account for the bulk of the balance except for **4d** and **4e**.
[b] The percentage was calculated by integrating HPLC chromatogram peaks at 230 nm.
[c] Unidentified oxidized compounds [M + 16] account for the balance.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Included are the detailed experimental procedure and spectroscopic data of **4a–k** and **5**; ^{1}H spectra and HRMS data sheet of **4a–k**; and ^{1}H, ^{31}P, and LRMS spectra of **5**.

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(40) **Typical Procedure for Cycloaddition Adducts 12a–k**
Reaction vessels were charged with resin **11** (100 mg) and NaN_3 (57.6 mg, 0.99 mmol, 10 equiv) followed by DMF (5 mL) and then CuI (3 mg) and agitate was initiated. Hünig's base (114 mg, 0.99 mmol, 10 equiv) was added, and, finally, the bromide (0.99 mmol, 10 equiv) was added. The resultant mixture was agitated for 1 d at r.t., heated to 50 °C for 3 h, then cooled. Then the resin was filtered and washed with DMF (2×), DMF–H_2O (1:1, 2×), DMF (2×), CH_2Cl_2 (3×), Et_2O (2×) and dried in vacuo.
General Procedure for the Synthesis of Alcohols 4a–k
Resin (100 mg) was heated at 80 °C with DCE–anhyd MeOH (1:1, 8 mL) in the presence of PPTS (5 mg) for 8 h. The liquid phase was collected, the resin was rinsed with CH_2Cl_2, and the washings were combined and sequentially washed with sat. aq $NaHCO_3$, H_2O, brine, dried over $MgSO_4$, concentrated, and purified by silica gel column chromatography to give **4a–k**.
Compound **4a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (s, 3 H), 2.35 (t, *J* = 8.4 Hz, 2 H), 2.81–2.85 (m, 2 H), 4.10 (d, *J* = 7.6 Hz, 2 H), 5.34–5.38 (m, 1 H), 5.48 (s, 2 H), 7.20 (s, 1 H), 7.21–7.27 (m, 2 H), 7.32–7.40 (m, 3 H) ppm. LRMS [M + H$^+$]: *m/z* = 258.2. HRMS [M$^+$]: *m/z* calcd for $C_{15}H_{19}N_3O$: 257.1528; found: 257.1529.
Compound **4b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.63 (s, 3 H), 2.31 (t, *J* = 8.0 Hz, 2 H), 2.81 (t, *J* = 8.0 Hz, 2 H), 4.10 (d, *J* = 6.8 Hz, 2 H), 5.33 (t, *J* = 6.8 Hz, 1 H), 5.58 (s, 2 H), 7.32–7.35 (m, 3 H), 8.14 (d, *J* = 10.8 Hz, 2 H) ppm. LRMS [M + H$^+$]: *m/z* = 302.2. HRMS [M$^+$]: *m/z* calcd for $C_{15}H_{18}N_4O_3$: 302.1379; found: 302.1375.
Compound **4c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 3 H), 1.66 (s, 3 H), 2.33 (t, *J* = 8.0 Hz, 2 H), 2.80 (t, *J* = 7.6 Hz, 2 H), 4.10 (d, *J* = 6.8 Hz, 2 H), 5.37 (t, *J* = 6.8 Hz, 1 H), 5.43 (s, 2 H), 7.16–7.20 (m, 3 H), 7.36 (d, *J* = 8.4 Hz, 2 H) ppm. LRMS [M + H$^+$]: *m/z* = 314.2. HRMS [M$^+$]: *m/z* calcd for $C_{19}H_{27}N_3O$: 313.2154; found: 313.2154.
Compound **4d**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23 (d, *J* = 6.8 Hz, 6 H), 1.69 (s, 3 H), 2.35 (t, *J* = 8.4 Hz, 2 H), 2.83 (t, *J* = 8.4 Hz, 2 H), 2.87–2.94 (m, 1 H), 4.10 (d, *J* = 6.8 Hz, 2 H), 5.30 (t, *J* = 8.0 Hz, 1 H), 5.44 (s, 2 H), 7.16–7.26 (m, 4 H) ppm. LRMS [M + H$^+$]: *m/z* = 300.2. HRMS [M$^+$]: *m/z* calcd for $C_{18}H_{25}N_3O$: 299.1998; found: 299.2002.
Compound **4e**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (s, 3 H), 2.35 (t, *J* = 8.0 Hz, 2 H), 2.83 (t, *J* = 8.0 Hz, 2 H), 4.11 (d, *J* = 6.8 Hz, 2 H), 5.38 (t, *J* = 8.0 Hz, 1 H), 5.45 (s, 2 H), 7.02–7.09 (m, 2 H), 7.21–7.26 (m, 3 H) ppm. LRMS [M + H$^+$]: *m/z* = 276.2. HRMS [M$^+$]: *m/z* calcd for $C_{15}H_{18}FN_3O$: 275.1434; found: 275.1431.
Compound **4f**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (s, 3

H), 2.36 (t, *J* = 8.0 Hz, 2 H), 2.84 (t, *J* = 8.0 Hz, 2 H), 4.11 (d, *J* = 6.8 Hz, 2 H), 5.38 (t, *J* = 8.0 Hz, 1 H), 5.48 (s, 3 H), 6.91 (d, *J* = 9.2 Hz, 1 H), 7.02–7.05 (m, 2 H), 7.30–7.35 (m, 1 H) ppm. LRMS [M + H$^+$]: *m/z* = 276.2. HRMS [M$^+$]: *m/z* calcd for $C_{15}H_{18}FN_3O$: 275.1434; found: 275.1437.

Compound **4g**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.69 (s, 3 H), 2.36 (t, *J* = 8.0 Hz, 2 H), 2.83 (t, *J* = 8.0 Hz, 2 H), 4.11 (d, *J* = 6.8 Hz, 2 H), 5.38 (t, *J* = 8.0 Hz, 1 H), 5.54 (s, 2 H), 7.08–7.37 (m, 4 H) ppm. LRMS [M + H$^+$]: *m/z* = 276.2. HRMS [M$^+$]: *m/z* calcd for $C_{15}H_{18}FN_3O$: 275.1434; found: 275.1429.

Compound **4h**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.71 (s, 3 H), 2.38 (t, *J* = 8.0 Hz, 2 H), 2.86 (t, *J* = 8.0 Hz, 2 H), 4.12 (t, *J* = 6.8 Hz, 2 H), 5.39 (t, *J* = 8.0 Hz, 1 H), 5.52 (s, 2 H), 7.10–7.25 (m, 4 H), 7.40 (t, *J* = 8.0 Hz, 1 H) ppm. LRMS [M + H$^+$]: *m/z* = 341.2. HRMS [M$^+$]: *m/z* calcd for $C_{16}H_{18}F_3N_3O_2$: 341.1350; found: 341.1359.

Compound **4i**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.67 (s, 3 H), 2.35 (t, *J* = 8.0 Hz, 2 H), 2.83 (t, *J* = 8.0 Hz, 2 H), 4.11 (d, *J* = 6.8 Hz, 2 H), 5.39 (t, *J* = 8.0 Hz, 1 H), 5.52 (s, 2 H), 7.18–7.27 (m, 4 H) ppm. LRMS [M + H$^+$]: *m/z* = 341.2. HRMS [M$^+$]: *m/z* calcd for $C_{16}H_{18}F_3N_3O_2$: 341.1350; found: 341.1357.

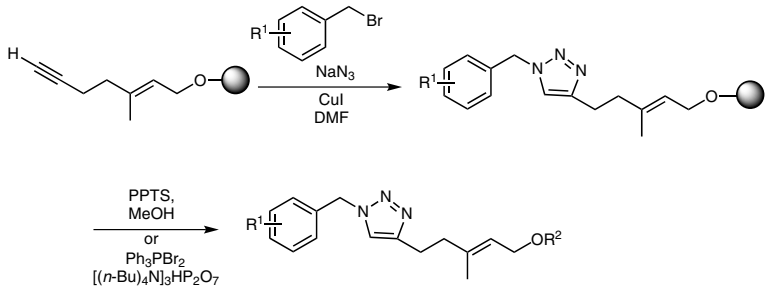
Compound **4j**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.69 (s, 3H), 2.36 (t, *J* = 8.0Hz, 2H), 2.85 (t, *J* = 8.0Hz, 2H), 4.11 (d, *J* = 6.8 Hz, 2 H), 5.38 (t, *J* = 8.0 Hz, 1 H), 5.53 (s, 2 H), 7.29 (s, 1 H), 7.47–7.50 (m, 2 H), 7.61–7.63 (m, 1 H) ppm. LRMS [M + H$^+$]: *m/z* = 283.2. HRMS [M$^+$]: *m/z* calcd for $C_{16}H_{18}N_4O$: 282.1480; found: 282.1482.

Compound **4k**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.70 (s, 3 H), 2.38 (t, *J* = 8.0 Hz, 2 H), 2.87 (t, *J* = 8.0 Hz, 2 H), 4.12 (d, *J* = 6.8 Hz, 2 H), 5.38 (t, *J* = 8.0 Hz, 1 H), 5.56 (s, 2 H), 5.56 (s, 2 H), 7.26 (s, 1 H), 7.31 (d, *J* = 12.0 Hz, 2 H), 7.65 (d, *J* = 12.0 Hz, 2 H) ppm. LRMS [M + H$^+$]: *m/z* = 283.2. HRMS [M$^+$]: *m/z* calcd for $C_{16}H_{18}N_4O$: 282.1480; found: 282.1487.

Procedure for the Synthesis of (*E*)-5-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-3-methylpent-2-en-1-yl Diphosphate (5)

The vacuum-dried resin **12a** (100 mg, 0.09 mmol) was suspended in CH_2Cl_2 (2 mL), Ph_3PBr_2 (84 mg, 0.19 mmol) was added, and the slurry was stirred at r.t. under N_2 for 4 h. Tris[tetra(*n*-butyl)ammonium]hydrogen diphosphate (388 mg, 0.39 mmol) in anhyd MeCN (3 mL) was added and the reaction mixture was stirred at r.t. for 4 h under N_2. The heterogeneous filtrate was concentrated, and the residue was suspended in 25 mM NH_4HCO_3 (4 mL) and extracted with Et_2O (5 mL, 3×). The aqueous phase was applied to an NH_4^+ form DOWEX-AW-50 ion-exchange column (50 mL of resin) and eluted with four-column volume 25 mM NH_4HCO_3 buffer. The aqueous phase was lyophilized to obtain the crude diphosphate **5** as a white solid, which was dissolved in a minimum volume of 25 mM NH_5CO_3 buffer and purified by RP-HPLC (Varian Dynamax, 10 μm, 300 Å, C-4 (10 mm × 250 mm) column with a gradient mobile phase: 25 mM NH_5AcO and MeCN.[15] The product collected between 5.2–5.6 min was lyophilized to obtain compound **5** (9.0 mg, 19% in 2 steps). ^{1}H NMR (400 MHz, D_2O): δ = 1.44 (s, 3 H), 2.10 (t, *J* = 7.6 Hz, 2 H), 2.57 (t, *J* = 7.6 Hz, 2 H), 4.15 (t, *J* = 6.8 Hz, 2 H), 5.11 (t, 6.4 Hz, 1 H), 5.28 (s, 2 H), 7.03–7.05 (m, 1 H), 7.14–7.20 (m, 2 H), 7.50 (s, 1 H) ppm. ^{31}P NMR (D_2O, 161.8 MHz): δ = –6.80 (d, *J* = 21.36 Hz, 1 P), –10.40 (d, *J* = 21.36 Hz, 1 P). LRMS [M + H$^+$]: *m/z* = 418.0.

(41) X-ray crystal data of the structure **4l** have been deposited at the Cambridge Crystallographic Data Center with the deposition number CCDC 871186.



graphical abstract