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PAPER

Pyridoxine-derived bicyclic aminopyridinol antioxidants: synthesis and their antioxidant activities†

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A few facile synthetic pathways for bicyclic aminopyridinol antioxidants are presented. Attachment of a long alkyl chain to the bicyclic pyridinol scaffold was established using ester linkage. Non-substituted pyrrolopyridinols and 1,3-oxazine-fused pyridinols were also synthesized as novel antioxidant scaffolds. Antioxidant activities were measured by a radical clock method and new compounds prepared are comparable to the best bicyclic aminopyridinol antioxidants.

Introduction

Since the introduction of mono- and bicyclic aminopyridinol antioxidants which typically possess better antioxidant activity than α-tocopherol (α-TOH), nature's most active chain-breaking antioxidant,[1] there have been an increasing number of synthetic approaches to this novel scaffold (Fig. 1).[2] The electronic effect of a ring nitrogen increases the ionization potential (IP) of these compounds and greatly improves the air-stability of aminopyridinols compared to the analogous phenolic compounds. Nitrogen ring substitution therefore addresses one of the major issues in the development of potent antioxidants.[3]

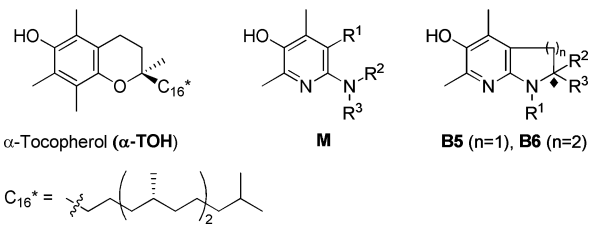

Fig. 1 Structures of α-tocopherol and mono- and bicyclic aminopyridinols.

Various modifications on the aminopyridinol scaffold have been made and relevant synthetic methods developed. In general, monocyclic aminopyridinols showed weaker antioxidant activities than bicyclic analogues, but the former are still excellent chain-breaking antioxidants. Many of these compounds have antioxidant activity several times better than α-TOH.[2g] In addition, some monocyclic derivatives are such good co-antioxidants that they can spare co-existing endogenous antioxidants such as uric acid to maximize the protective effect against oxidative stress.[2j] Among the bicyclic aminopyridinols are a group of derivatives that have lipophilic side chains. These hydrophobic analogues showed significantly superior antioxidant properties to α-TOH in that (i) they have 10–80 times higher rate constant (k_{inh}) for inhibition of the radical chain reaction, (ii) they spare endogenous α-TOH from consumption, and (iii) they do not propagate α-TOH-mediated peroxidation processes.[2c] Importantly, the most advanced analogue of this kind, N-TOH (**B6**, $R^1 = R^2 = Me$, $R^3 = C_{16}$*), showed further characteristics which could render it a good *in vivo* antioxidant.[2d] It was suggested that N-TOH can diffuse 1.5-times faster than α-TOH to the site of a lipid peroxyl radical in a biological membrane, and that it binds better than α-TOH to the tocopherol transfer protein (hTTP).[2d]

We have recently reported a significantly improved synthetic pathway to the aminopyridinols where pyridoxine·HCl (**1**, one of the constituents of vitamin B_6) is the starting material (Fig. 2). As a result, bicyclic aminopyridinols, such as naphthyridinol **2**[2j] and pyrrolopyridinol **3**,[2k] were easily synthesized in large quantity starting from **1**. In addition, a new series of monocyclic analogue **4**[2j] was derived from **1**. In the current study, we report on the preparation of another series from this versatile antioxidant scaffold. We describe here the synthesis of various types of pyridoxine-derived bicyclic aminopyridinols along with their antioxidant activities.

Results and discussion

Antioxidant activities of phenolic compounds are largely dependent on the degree of orbital overlap between the oxygen radical center and the lone-pair orbital of a *para*-substituted heteroatom.[3a]

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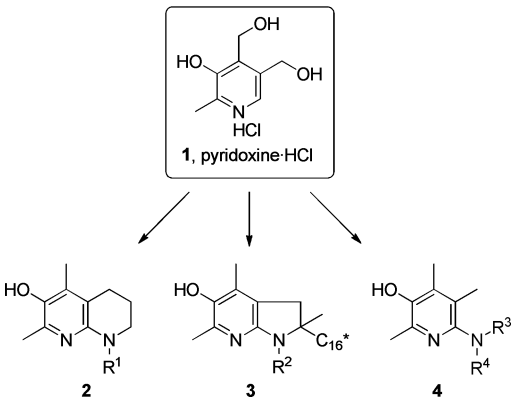
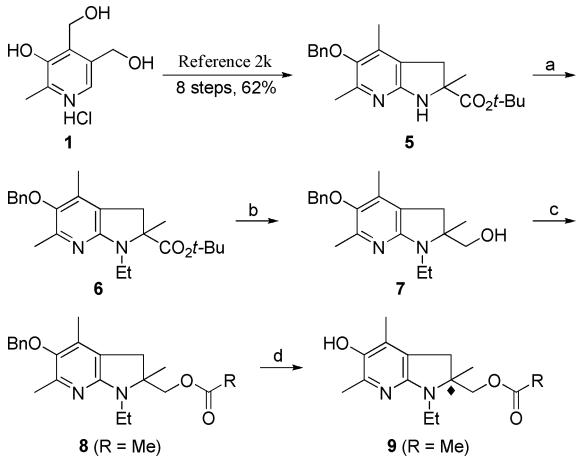


Fig. 2 Pyridoxine·HCl, a highly efficient natural source for aminopyridinols (R^1, R^2, R^3, R^4 = H or alkyl group).

Therefore, the activity can be modulated by the pattern of the substituents attached to the *para*-substituted heteroatom since this substitution can determine the degree of orbital overlap. For example, better antioxidant activities of bicyclic compounds than their monocyclic analogues in both the phenol and aminopyridinol series has been attributed to the favorable orbital overlap due to the stereoelectronic effect of the bicyclic geometry. Furthermore, within the bicyclic aminopyridinol system, it has been reported that the geometry of five-membered pyrrolidine-fused system **B5** is more favorable for orbital overlap than that of the six-membered piperidine-fused system **B6**. The improved overlap results in a two to three-fold better antioxidant activity for pyrrolidines than is observed for the piperidines.[1b]

While substitutions at *para*-position dramatically alter this orbital overlap, substitutions on the α-carbon (*i.e.*, *C*(◆)-position in **B6**) to the *para*-nitrogen do not seem to significantly modify the stereoelectronic effect in the aminopyridinol system. In piperidine-fused aminopyridinols **B6**, the antioxidant activities, reflected in k_{inh}, vary only <20% depending on R^2 and R^3 groups.[2d] On the other hand, it is well documented that the stereochemistry of the quaternary carbon center in α-TOH (equivalent to C(◆)-position in **B6**) where C_{16}*-chain is attached to chromanol system is important for its *in vivo* activity[4] because it is crucial for the binding affinity of α-TOH to hTTP. It is, of course, uncertain that a C_{16}-isoprenoid chain will lead to the maximal activity for the aminopyridinols. For example, a class of bicyclic aminopyridinol **B6** (R^1 = $CH_2(CH_2)_{14}CH_3$, R^2 = R^3 = H) with a simple linear C_{16}-chain connected to piperidine ring nitrogen rather than to the *C*(◆)-position also showed excellent antioxidant activities. Although this compound remains untested on its binding affinity to hTTP, it spared endogenous α-TOH and did not promote tocopherol-mediated lipid peroxidation in an isolated human LDL particle.[2c]

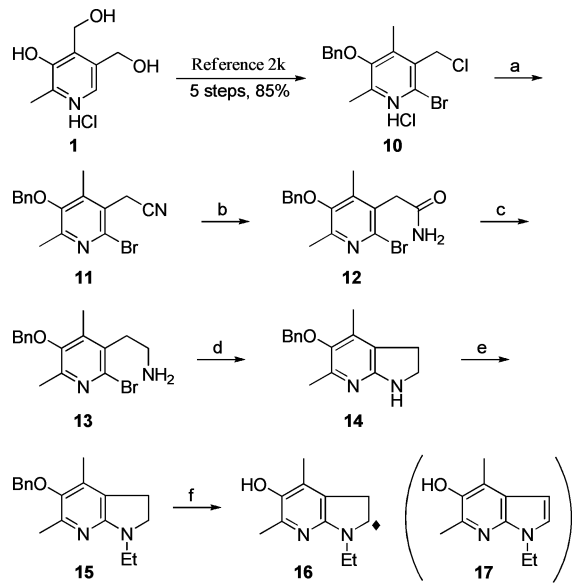
We have first explored the possibility that we can attach a lipophilic side chain in a way to avoid a long synthetic procedure for the C_{16}-isoprenoid unit (C_{16}*). To this end, a fatty acid (RCO_2H) was attached to bicyclic system *via* a simple esterification (Scheme 1). This neopentyl-type ester bond in **9** should be stable toward hydrolysis, thereby providing lipophilicity in cellular and physiological environments. As shown in Scheme 1, by putting an acetyl group as a representative acyl group, we have demonstrated that we can easily elongate the lipophilic moiety of **9**. As for the



Scheme 1 *Reagents and conditions* (a) CH_3CHO, AcOH, $NaBH_3CN$, MeOH, rt, 2 h, 84%; (b) DIBAL-H, CH_2Cl_2, 0 °C, 4 h, 92%; (c) Ac_2O, pyridine, DMAP, rt, 0.5 h, 96%; (d) H_2, Pd/C, MeOH, rt, 3 h, 93%.

intermediate **5**, we have developed and reported a high-yielding synthesis starting from pyridoxine·HCl (**1**).[2k] The NH group of **5** was ethylated by reductive alkylation for solubility reason to give **6** in 84% yield. Unlike the substitution at the *C*(◆)-position, *N*-alkyl substitution (*N*-ethyl *vs.* *N*-methyl) would not have significant stereoelectronic effect on antioxidant activity. *t*-Butyl ester group of **6** was reduced to alcohol **7** with DIBAL-H in 92% yield. The alcohol group in **7** was then acetylated to afford **8** (96%) where R = CH_3 is a representative fatty acid (RCO_2H). A lipophilic side chain in the form of an *O*-acyl group should provide solubility in bulk solvents. Catalytic hydrogenolysis removed benzyl group to give the dialkyl model compound **9** in 93% yield.

Next, in order to compare the stereoelectronic effect, **16** having no alkyl substitution at *C*(◆)-position was synthesized (Scheme 2).

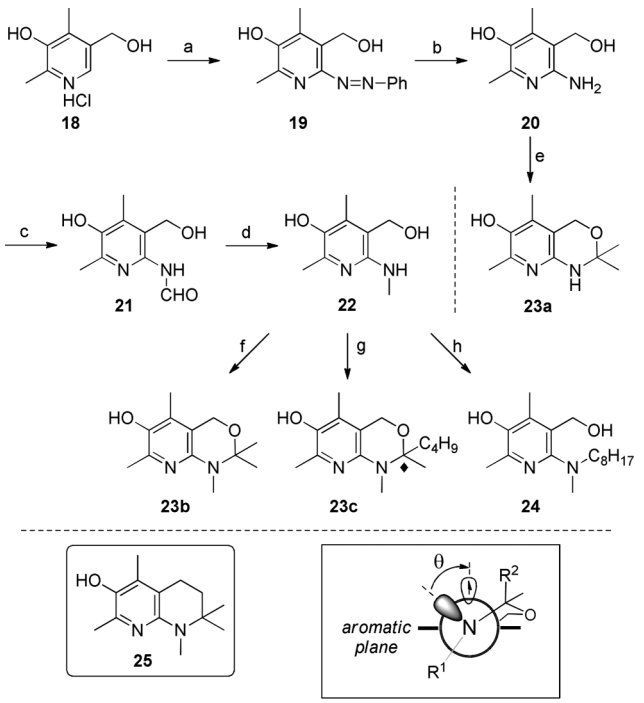


Scheme 2 *Reagents and conditions* (a) KCN, acetone-H_2O, reflux, 1 h, 98%; (b) KOH, EtOH, reflux, 12 h, 88%; (c) BH_3-THF, THF, reflux, 12 h, 40%; (d) CuOAc, K_3PO_4, diethyl salicylamide, DMF, 40 °C, 15 h; (e) CH_3CHO, AcOH, $NaBH_3CN$, MeOH, rt, 2 h, 63% for two steps; (f) H_2, Pd/C, MeOH, 3 h, rt, 93%.

Although the synthesis of *N*-methyl version of **16** was already reported using 2-nitro-*m*-xylene as an oxygenating agent,[1b,2a] we report here a new synthesis of **16** starting from pyridoxine·HCl (**1**). We chose to prepare the bicyclic aminopyridinol **16** containing the *N*-ethyl group for better solubility and structural homology to aminopyridinol **9**. Displacement of chloride in **10**, which could be readily prepared from pyridoxine·HCl (**1**) according to the synthetic method developed by us,[2k] from the nucleophilic cyanide anion in refluxing wet acetone to give **11** in 98% yield. Hydrolysis of the nitrile group in **11** with ethanolic KOH solution under reflux conditions afforded the primary amide **12** in 88% yield. The primary amine **13** could be prepared by borane reduction of **12** in refluxing THF but only a modest yield (40%) was obtained. Pyrrolidine ring formation was accomplished by intramolecular Cu(I)-catalyzed amination of **13** to give **14**, and the reductive ethylation that followed afforded **15** in overall 63% yield for these two steps. The benzyl protecting group in **15** was finally removed by hydrogenolysis to give the *C*(◆)-unsubstituted pyrrolopyridinol **16** in 93% yield. We found that **16** was unstable in air as suggested by its *N*-methyl analogue;[1b] about 7% of **16** was found to be rearomatized to **17** after several days. It clearly demonstrates its susceptibility toward benzylic oxidation/aromatization presumably through an electron transfer to molecular oxygen. The ionization potential (IP) of the *N*-methyl analogue was reported to be 152.3 kcal mol^{-1},[1b] low enough to react with molecular oxygen. *N*-Ethyl substitution in **16** should not change its IP significantly.

Our investigations of the stereoelectronic effect were extended in scope to consider 1,3-oxazine-annulated pyridinol **23** as a model structure to study the effect of the oxygen atom at the C(5′)-position on the orbital overlap between the pyridinoxyl radical and the *para*-substituted nitrogen atom (Scheme 3). Although a *C*(5′)-oxygen could not provide a direct electron donating effect on the aromatic ring, it still can exert stereoelectronic effect in structure **23**. For maximum orbital overlap, the lone pair of the *para*-nitrogen atom should be oriented perpendicular to the aromatic ring plane ($\theta = 0$)[3a,5] and this orbital overlap can be dramatically increased by bicyclic geometry. We envisioned that this favorable geometry could be easily induced by the *N*,*O*-ketal structure shown in **23** and it could serve as an attractive antioxidant scaffold to the tetrahydro-1,8-naphthyridinols **B6** because of the simpler synthetic approaches and the readily available starting material, 4-deoxypyridoxine·HCl (**18**)[2j] which in turn can be prepared from pyridoxine·HCl (**1**) in high yield. It turns out to be very convenient to install the quaternary substitution at *C*(◆)-position in the structure **23**.

For the synthesis of **23** (Scheme 3), **18** was coupled with diazotized aniline, then, the diazo group of **19** was reduced under catalytic hydrogenation conditions to form 6-amino-4-deoxypyridoxine (**20**) in 86% for two steps. Triformylation of **20** by acetic formic anhydride/formic acid followed by selective hydrolysis afforded the 6-formamido derivative **21** in 68% yield for two steps. The formamido group of **21** was reduced with borane-THF complex to give the *N*-methyl product **22** in 88% yield. Subsequent *N*,*O*-ketalization with 2,2-dimethoxypropane and 2-hexanone gave **23a** (70% from **20**), **23b** and **23c** (58% and 44% from **22**), respectively. Monocyclic derivative **24** was also prepared from **22** to examine the effects of the structural difference between monocyclic and bicyclic systems on the antioxidant activity.



Scheme 3 *Reagents and conditions* (a) $PhNH_2$, HCl, $NaNO_2$, NaOH, H_2O, 0 °C to rt, 1 h, 95%; (b) H_2, Pd/C, MeOH, rt, 6 h, 90%; (c) (i) Ac_2O, HCO_2H, 60 °C, 20 min then **19**, rt, 40 min, (ii) K_2CO_3, MeOH, 0 °C, 30 min, 68% for two steps; (d) BH_3–THF, THF, reflux, 4 h, 88%; (e) $(MeO)_2CMe_2$, *p*-TsOH, DMSO, rt, 20 h, 70%; (f) $(MeO)_2CMe_2$, *p*-TsOH, DMSO, rt, 20 h, 58%; (g) 2-hexanone, camphorsulfonic acid, DMSO, rt, 24 h, 44%; (h) *n*-$C_7H_{15}CHO$, $NaBH(OAc)_3$, AcOH, MeOH, rt, 3 h, 73%.

Antioxidant activities of selected compounds are shown in Table 1. The inhibition rate constants (k_{inh}) of the aminopyridinols, which correspond to the rate constant for H-atom transfer to the chain-carrying peroxyl radicals in lipid peroxidation, were measured by a peroxyl radical clock system based on the autoxidation of pentafluorobenzyl 13(*S*)-hydroxyoctadeca-6*Z*,9*Z*,11*E*-trienoate (PFB-13-HOTrE) (Fig. 3). This radical clock is similar to the linoleate peroxyl radical clocks previously described, but relies on the assistance of mass spectrometry for product analysis.[7] This clock is based on the competition between trapping the kinetically favored 8-hydroperoxy, 13(*S*)-6*Z*,9*Z*,11*E*-trienoate (8,13-*c*,*c*,*t*) *versus* peroxyl radical *β*-fragmentation that leads to other thermodynamically more stable products. This system gives good values of k_{inh} as has been established with antioxidants having known k_{inh} values.

Table 1 Rate constants (k_{inh}) measured by PFB-13-HOTrE radical clock[a,6]

Compounds	k_{inh} (10^7 $M^{-1}s^{-1}$)	$k_{inh}/k_{inh(\alpha\text{-TOH})}$
α-TOH	0.35	1.0
9	3.95 ± 0.72	11.3
16	3.79 ± 0.65	10.8
23c	3.07 ± 0.47	8.8
24	1.60 ± 0.19	4.6
25[b]	5.20 ± 0.78	14.9

[a] See experimental section for details. [b] **25** was prepared by the reported procedure,[2d] where its k_{inh} was reported.

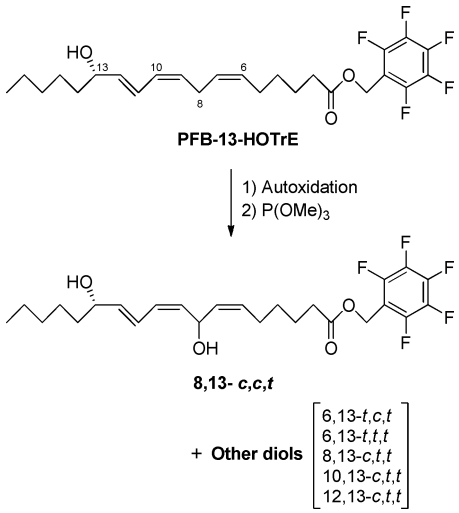


Fig. 3 The PFB-13-HOTrE radical clock.

The compound **23c** is a slightly poorer H-atom donor than the tetrahydro-1,8-naphthyridin-3-ol analogue **25**. Oxygen substitution at the 3-position and bulky dialkyl substitution (*C*(◆)-methylbutyl) in **23c** possibly decreases orbital overlap by distorting molecular geometry. On the other hand, **23c** showed about two-fold higher k_{inh} than its monocyclic counterpart **24**, confirming the notion of a favorable stereoelectronic effect. Although **24** possibly mimics the bicyclic geometry through a intramolecular hydrogen bond between the free OH group and *para*-nitrogen, the conformational analogy, if any, did not contribute to the antioxidant activity. Indeed, it is likely that an intramolecular hydrogen bond would be at the expense of the lone pair electron of the *para*-nitrogen and cause a larger dihedral angle (θ) to the pyridinoxyl radical. It is noteworthy, however, that the monocycle **24** and the bicycle **23c** are 5- and 10-fold better antioxidants than α-TOH, respectively, demonstrating the novelty of the scaffold.

Pyrrolopyridinols **9** and **16** showed interesting antioxidant activities. First, *C*(◆)-dialkylated **9** and non-alkylated **16** have similar k_{inh}. It suggested that both stereoelectronic and direct electron donating effect of *C*(◆)-substitution is less important in pyrrolopyridinol scaffold. Second, it seems that the ester linkage in **9** does not significantly perturb k_{inh}, demonstrating the usefulness of this elongation strategy. Finally, five-membered ring-fused pyrrolopyridinols appear to have less favorable orbital overlap compared to the six-membered ring-fused tetrahydro-1,8-naphthyridinols than we has been previously expecting.[1b] In fact, the (**9** and **16**) rate constants (k_{inh}) lie in close range to that of the representative tetrahydro-1,8-naphthyridinol **25**.

Conclusions

A facile approach to attach an alkyl chain to the pyrrolopyridinol antioxidants has been developed. Methods reported can be applied to the synthesis of the tetrahydro-1,8-naphthyridinol scaffold as well. In addition, we have introduced the synthesis of *C*(◆)-non substituted pyrrolopyridinols and 1,3-oxazine-fused series of bicyclic aminopyridinols as novel antioxidant scaffolds. These facile synthetic pathways and their excellent antioxidant activities demonstrate the versatility of the synthetic approaches and the possible range of application of these antioxidants.

Experimental

General

Unless noted otherwise, materials were purchased from commercial suppliers and used without further purification. Air- or moisture-sensitive reactions were carried out under an inert gas atmosphere. THF and CH_2Cl_2 were dried using a Solvent Purification System from Solvtek. Progress of reaction was monitored by thin-layer chromatography (TLC) using silica gel F_{254} plates. Purification of the products was performed by flash column chromatography using silica gel 60 (230–400 mesh) or a Biotage SP-1 system with indicated solvents. IR spectra were recorded on a Perkin Elmer Spectrum GX FT-IR spectrometer. NMR spectra were taken with a 300 MHz Bruker NMR spectrometer. Chemical shifts (δ) were expressed in ppm using solvent as an internal standard and coupling constant (J) in hertz. Melting points were taken on a Fischer-Jones melting point apparatus and are uncorrected. Low-resolution mass spectra (MS) were recorded on a Agilent Technologies Quadruple 6130 LC/MS. High-resolution mass spectra (HRMS) were recorded using the electrospray technique. ES-HRMS measurement were performed at Ohio State University.

***tert*-Butyl 5-benzyloxy-1-ethyl-2,4,6-trimethyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxylate (6).** To a solution of **5** (720 mg, 1.95 mmol) in methanol (20 mL) were added acetaldehyde (1.1 mL, 19.5 mmol) and acetic acid (0.56 mL, 9.75 mmol). After addition of sodium cyanoborohydride (613 mg, 9.75 mmol), the reaction mixture was stirred for 2 h at room temperature. Methanol was evaporated and the residue was basified with sat. Na_2CO_3 solution. Extraction of the mixture with EtOAc (100 mL×2) was performed. The combined organic solution was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (hexanes : EtOAc = 6 : 1) to give **6** (650 mg, 84%) as pale yellow oil. ^{1}H NMR (300 MHz, $CHCl_3$-*d*) δ 7.34–7.46 (m, 5H), 4.71 (s, 2H), 3.45–3.52 (m, 1H), 3.16–3.25 (m, 2H), 2.79 (d, 1H, J = 16.2 Hz), 2.39 (s, 3H), 2.08 (s, 3H), 1.52 (s, 3H), 1.43 (s, 9H), 1.27 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CHCl_3$-*d*) δ 173.7, 157.7, 146.9, 144.1, 137.6, 135.4, 128.4, 127.9, 117.1, 81.3, 75.0, 69.2, 38.6, 37.5, 27.9, 22.8, 19.2, 15.0, 12.6; IR (KBr) ν 2975, 2930, 1726, 1620, 1585, 1475, 1368, 1268, 1211, 1156, 1116, 1096, 1037, 1013, 846, 733, 697 cm^{-1}; MS (ESI+): *m*/*z* 397.3 $[M+H]^+$; HRMS calcd for $C_{24}H_{32}N_2O_3$ $[M+H]^+$ 397.2492, found 397.2492.

(5-Benzyloxy-1-ethyl-2,4,6-trimethyl-2,3-dihydro-1*H*-pyrrolo-[2,3-*b*]pyridin-2-yl)methanol (7). To a cooled (0 °C) solution of **6** (570 mg, 1.44 mmol) in anhydrous CH_2Cl_2 (20 mL) was added 1 M DIBAL-H in THF (1.58 mL, 1.58 mmol). After the mixture was stirred for 4 h at 0 °C, the mixture was quenched with a few drops of methanol and warmed up to room temperature. sat. Sodium potassium tartrate solution (20 mL) was added and the mixture was stirred for 1 h at room temperature. Extraction with CH_2Cl_2 (100 mL × 2) was carried out and the combined extracts were dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (hexanes : EtOAc = 2 : 1) to yield **7** (432 mg, 92%) as a pale yellow solid. m.p. 123 °C; ^{1}H NMR (300 MHz, $CHCl_3$-*d*) δ 7.35–7.49 (m, 5H), 4.72 (s, 2H), 3.67 (d, 1H, J = 11.1 Hz), 3.35–3.42 (m, 2H), 3.18–3.25 (m, 1H), 3.12 (d, 1H,

J = 16.2 Hz), 2.56 (d, 1H, J = 16.2 Hz), 2.40 (s, 3H), 2.11 (s, 3H), 1.29 (t, 3H, J = 7.2 Hz); 1.18 (s, 3H); ^{13}C NMR (75 MHz, $CHCl_3$-d) δ 157.8, 146.8, 144.5, 137.5, 136.1, 128.5, 127.9, 127.8, 118.1, 75.0, 66.7, 66.5, 35.5, 35.2, 21.4, 19.2, 15.3, 12.7; IR (KBr) ν 3386, 3032, 2965, 2927, 2868, 1623, 1579, 1486, 1433, 1399, 1367, 1215, 1095, 1049, 989, 739, 697 cm^{-1}; MS (ESI+): m/z 327.2 $[M+H]^+$; HRMS calcd for $C_{20}H_{26}N_2O_2$ $[M+H]^+$ 327.2073, found 327.2074.

(5-Benzyloxy-1-ethyl-2,4,6-trimethyl-2,3-dihydro-1*H*-pyrrolo-[2,3-*b*]pyridin-2-yl)methyl acetate (8). To a solution of **7** (223 mg, 0.68 mmol) in pyridine (3 mL) was added 4-dimethylaminopyridine (17 mg, 0.14 mmol) and acetic anhydride (77 μL, 0.82 mmol) at room temperature. After the mixture was stirred for 30 min at room temperature, the mixture was diluted with EtOAc (200 mL) and successively washed with water (50 mL) and brine (50 mL). The EtOAc solution was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (hexanes : EtOAc = 3 : 1) to yield **8** (240 mg, 96%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CHCl_3$-d) δ 7.32–7.50 (m, 5H), 4.71 (s, 2H), 4.07 (dd, 2H, J = 15.8, 11.4 Hz), 3.33 (q, 2H, J = 8.1 Hz), 2.94 (d, 1H, J = 16.2 Hz), 2.67 (d, 1H, J = 16.2 Hz), 2.39 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H), 1.30 (s, 3H); 1.23 (t, 3H, J = 8.1 Hz); ^{13}C NMR (75 MHz, $CHCl_3$-d) δ 170.9, 157.3, 146.7, 143.9, 137.6, 135.7, 128.5, 127.9, 127.8, 117.3, 75.0, 68.4, 64.2, 36.8, 35.4, 22.2, 20.8, 19.2, 15.3, 12.6; IR (KBr) ν 3031, 2967, 2928, 1743, 1620, 1582, 1477, 1429, 1398, 1371, 1324, 1224, 1094, 1040, 990, 725, 730 cm^{-1}; MS (ESI+): m/z 369.2 $[M+H]^+$; HRMS calcd for $C_{22}H_{28}N_2O_3$ $[M+H]^+$ 369.2178, found 369.2170.

(1-Ethyl-5-hydroxy-2,4,6-trimethyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)methyl acetate (9). To a solution of **8** (125 mg, 0.34 mmol) in methanol (5 mL) was added palladium (10% on activated carbon, 10 mg). The mixture was stirred with hydrogen balloon for 3 h at room temperature. The solid in the reaction mixture was filtered through Celite pad and the filtrated was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **9** (88 mg, 93%) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.36 (s, 1H), 3.99 (dd, 2H, J = 30.9, 11.1 Hz), 3.15 (q, 2H, J = 6.9 Hz), 2.82 (d, 2H, J = 15.9 Hz), 2.55 (d, 2H, J = 15.9 Hz), 2.17 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.17 (s, 3H); 1.08 (t, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.7, 155.2, 141.2, 140.4, 132.3, 117.2, 68.0, 64.3, 36.6, 35.4, 21.9, 21.0, 19.8, 15.7, 13.0; IR (KBr) ν 3392, 2971, 2932, 1741, 1669, 1585, 1431, 1377, 1353, 1323, 1232, 1095, 1044, 991, 752 cm^{-1}; MS (ESI+): m/z 279.2 $[M+H]^+$; HRMS calcd for $C_{15}H_{22}N_2O_3$ $[M+H]^+$ 279.1709, found 279.1704.

3-Benzyloxy-6-bromo-5-cyanomethyl-2,4-dimethylpyridine (11). To a suspension of **10** (300 mg, 0.796 mmol) in acetone (2.5 mL) was added a solution of potassium cyanide (363 mg, 5.568 mmol) in water (2 mL). The reaction mixture was refluxed for 1 h. The mixture was diluted with chloroform (30 mL) and water (5 mL) and the aqueous layer was extracted with chloroform (20 mL×2). The organic solution was washed with brine and dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (hexanes : EtOAc = 4 : 1) to give **11** (258 mg, 98%) as a light yellow solid. m.p. 87–88 °C; ^{1}H NMR (300 MHz, $CHCl_3$-d) δ 7.42–7.44 (m, 5H), 4.83 (s, 2H), 3.85 (s, 2H), 2.51 (s, 3H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, $CHCl_3$-d) δ 154.2, 152.2, 142.9, 137.7, 136.3, 129.2, 129.1, 128.5, 125.3, 116.1, 75.7, 22.0, 19.9, 14.1; IR (KBr) ν 3032, 2923, 2253, 1575, 1497, 1440, 1408, 1366, 1249 m, 1215, 1189, 1086, 965, 935, 910, 757, 700 cm^{-1}; MS (ESI+): m/z 331.0 $[M+H]^+$; HRMS calcd for $C_{16}H_{16}BrN_2O$ $[M+H]^+$ 331.0446, found 331.0443.

2-(3-Benzyloxy-6-bromo-2,4-dimethylpyridin-5-yl)acetamide (12). To a solution of **11** (1.0 g, 3.02 mmol) in ethanol (40 mL) was added potassium hydroxide (997 mg, 15.10 mmol) and the resulting mixture was refluxed for 12 h. After concentration of the mixture, the residue was diluted with water (20 mL) and extracted with chloroform (30 mL×3). The combined extracts were dried over $MgSO_4$ and concentrated to afford **12** (928 mg, 88%) as a yellow solid. For analytical sample, silica gel column chromatography ($CHCl_3$: MeOH = 20 : 1) was carried out to give a light yellow solid. m.p. 191–195 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.38–7.50 (m, 6H), 7.05 (s, 1H), 4.82 (s, 2H), 3.63 (s, 2H), 2.37 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.2, 151.5, 151.1, 143.5, 138.4, 137.0, 131.2, 128.8, 128.7, 128.6, 74.7, 39.0, 19.2, 13.8; IR (KBr) ν 3375, 3197, 2922, 1667, 1618, 1399, 1363, 1247, 1218, 1183, 1095, 977, 939, 841, 730, 695 cm^{-1}; MS (ESI+): m/z 349.0 $[M+H]^+$; HRMS calcd for $C_{16}H_{18}BrN_2O_2$ $[M+H]^+$ 349.0552, found 349.0539.

5-Aminoethyl-3-benzyloxy-6-bromo-2,4-dimethylpyridine (13). To a suspension of **12** (500 mg, 1.43 mmol) in dry THF (10 mL) was added BH_3-THF complex (1.0 M in THF, 5.72 mL, 5.72 mmol) at room temperature. The reaction mixture was refluxed for 12 h and cooled down to room temperature. After quenching the reaction mixture with a few drops of water, sat. $NaHCO_3$ solution was added to the mixture. The resulting mixture was stirred for 10 min and extracted with EtOAc (50 mL×3). The combined extracts were washed with brine and dried over $MgSO_4$. After filtration and concentration, the residue was purified over silica gel column chromatography (CH_2Cl_2 : MeOH = 20 : 1) to afford **13** (192 mg, 40%) as a sticky caramel. ^{1}H NMR (300 MHz, $CHCl_3$-d) δ 7.35 (s, 5H), 4.74 (s, 2H), 3.67 (br s, 2H), 2.90–3.09 (m, 4H), 2,42 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (75 MHz, $CHCl_3$-d) δ 152.9, 152.4, 142.8, 138.4, 136.4, 131.1, 129.1(×2), 129.0(×2), 128.5, 75.5, 47.4, 32.0, 19.8, 13.8; IR (KBr) ν 3360, 3032, 2925, 2871, 1729, 1574, 1454, 1401, 1367, 1249, 1215, 1124, 950, 738, 698 cm^{-1}; MS (ESI+): m/z 335.1 $[M+H]^+$; HRMS calcd for $C_{16}H_{20}BrN_2O$ $[M+H]^+$ 335.0759, found 335.0751.

5-Benzyloxy-1-ethyl-4,6-dimethyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridine (15). To a mixture of copper(I) acetate (2.2 mg, 0.018 mmol), *N*,*N*-diethylsalicylamide (14 mg, 0.072 mmol) and potassium phosphate tribasic (153 mg, 0.72 mmol) was added a solution of **13** (120 mg, 0.36 mmol) in dry DMF (4 mL). After the reaction mixture was stirred for 15 h at 40 °C, 1 M NaOH (1 mL) was added to the mixture. The mixture was diluted with EtOAc (100 mL) and water (10 mL) and the organic layer was washed with water (10 mL×4). The organic layer was dried over $MgSO_4$ and concentrated to afford crude **14**.

The crude **14** was dissolved in methanol (10 mL) were added acetaldehyde (0.2 mL, 3.60 mmol) and acetic acid (0.1 mL, 1.80 mmol). After addition of sodium cyanoborohydride (113 mg, 1.80 mmol), the reaction mixture was stirred for 2 h at room temperature. Methanol was evaporated and the residue was basified with sat. Na_2CO_3 solution. After extraction of the mixture with EtOAc (50 mL×2), the combined organic solution was washed

with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (hexanes : EtOAc = 5 : 1) giving **15** (64 mg, 63% from **13**) as a pale yellow oil. ^{1}H NMR (300 MHz, $CHCl_3$-*d*) δ 7.27–7.49 (m, 5H), 4.74 (s, 2H), 3.44 (t, 2H, J = 8.4 Hz), 3.36 (q, 2H, J = 7.2 Hz), 2.84 (t, 2H, J = 8.4 Hz), 2,38 (s, 3H), 2.11 (s, 3H), 1.19 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CHCl_3$-*d*) δ 152.9, 152.4, 142.8, 138.4, 136.4, 131.1, 129.1(×2), 129.0(×2), 128.5, 75.5, 47.4, 32.0, 19.8, 13.8; IR (KBr) ν 3441, 2923, 2852, 1735, 1581, 1495, 1456, 1354, 1252, 1216, 1090, 731, 696 cm^{-1}; MS (ESI+): *m/z* 283.2 $[M+H]^+$; HRMS calcd for $C_{18}H_{23}N_2O$ $[M+H]^+$ 283.1810, found 283.1798.

1-Ethyl-4,6-dimethyl-2,3-dihydro-1*H*-pyrrolo[2,3-*b*]pyridin-5-ol (16). To a solution of **15** (55 mg, 0.19 mmol) in methanol (1 mL) was added palladium (10% on activated carbon, 5 mg). The mixture was stirred with hydrogen balloon for 3 h at room temperature. The solid in the reaction mixture was filtered through Celite pad and the filtrated was filtered again with syringe filter (Advantec® JP050AN). The filtrate was concentrated to give **16** (34 mg, 93%) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.49 (s, 1H), 3.27 (t, 2H, J = 8.1 Hz), 3.16 (q, 2H, J = 7.2 Hz), 2.74 (t, 2H, J = 8.1 Hz), 2.19 (s, 3H), 2.01 (s, 3H), 1.06 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.8, 141.8, 140.4, 132.4, 120.1, 50.0, 41.2, 24.7, 19.7, 13.1, 12.6; IR (KBr) ν 3392, 2993, 2922, 2848, 1593, 1453, 1380, 1119, 1050, 918, 761 cm^{-1}; MS (ESI+): *m/z* 193.1 $[M+H]^+$; HRMS calcd for $C_{11}H_{17}N_2O$ $[M+H]^+$ 193.1341, found 193.1340.

5-(Hydroxymethyl)-2,4-dimethyl-6-(phenyldiazenyl)pyridin-3-ol (19). In a beaker equipped with a mechanical stirrer and pH electrode, **18** (3 g, 15.82 mmol) was dissolved in water (40 mL) and cooled in an iced bath. In a separated Erlenmeyer flask, aniline (1.59 mL, 17.40 mmol) was dissolved in 6 M HCl (15.9 mL) at room temperature, and then cooled in iced bath. A cooled solution of sodium nitrite (1.2 g, 17.40 mmol) in water (5 mL) was added in small portions to the aniline solution. This diazotized aniline solution was then added in small portions to the chilled solution of **18**. After each addition, the pH of the reaction mixture was quickly adjusted to maintain pH 8 by addition of 2.5 M NaOH. After the addition of the diazotized aniline solution, the reaction mixture was allowed to warm to room temperature and stirred for 1 h. After the reaction was completed, the mixture was placed in an iced bath for 12 h. The solid precipitated was collected by filtration to afford **19** (3.86 g, 95%) as a red solid. m.p. 79–80 °C; ^{1}H NMR (300 MHz, $CHCl_3$-*d*) δ 7.13–7.36 (m, 5H), 4.83 (s, 2H), 2.38 (s, 3H), 2.09 (s, 3H); IR (KBr) ν 3240, 3059, 2958, 1599, 1557, 1509, 1445, 1412, 1226, 1112, 1000, 920, 763, 735, 690 cm^{-1}; MS (ESI+): *m/z* 258.1 $[M+H]^+$; HRMS calcd for $C_{14}H_{16}N_3O_2$ $[M+H]^+$ 258.1243, found 258.1225.

6-Amino-5-(hydroxymethyl)-2,4-dimethylpyridin-3-ol (20). To a solution of **19** (4.0 g, 15.54 mmol) in methanol (400 mL) was added 10% palladium on activated carbon (400 mg). The mixture was stirred for 6 h at room temperature under H_2 atmosphere (balloon). After filtration of the reaction mixture, 80% volume of the filtrate was evaporated to give a suspension. Et_2O (200 mL) was added to the residue and stirred for 1 h at room temperature. The solid precipitate was collected by filtration and washed with Et_2O to give **20** (2.35 g, 90%) as a white solid. m.p. 209–210 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (s, 1H), 4.97 (s, 2H), 4.77 (s, 1H), 4.35 (d, 2H, J = 4.8 Hz), 2.16 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.8, 142.7, 141.4, 135.2, 116.5, 56.9, 19.5, 12.1; IR (KBr) ν 3446, 3380, 2922, 2848, 1618, 1592, 1566, 1382, 1307, 1241, 1134, 1005, 774 cm^{-1}; MS (ESI+): *m/z* 169.1 $[M+H]^+$; HRMS: calcd for $C_8H_{12}N_2O_2$ $[M+H]^+$ 169.0972, found 169.0974.

***N*-(5-Hydroxy-3-(hydroxymethyl)-4,6-dimethylpyridin-2-yl)formamide (21)**

21-1. 6-Formamido-5-((formyloxy)methyl)-2,4-dimethylpyridin-3-yl formate (21-1). To formic acid (8.96 mL, 237.6 mmol) was added acetic anhydride (22.4 mL, 237.6 mmol) and the mixture was stirred for 20 min at 60 °C. This mixture was cooled down to room temperature then, **20** (5.0 g, 29.7 mmol) was added. It was stirred for 30 min at room temperature then, 10 min at 60 °C. The reaction mixture was slowly transferred to ice-cold sat. $NaHCO_3$ solution. After 5 min stirring in ice-bath, the resulting slurry was filtered was washed with ice-cold water. Filter cake was dried under vacuum to give the triformyl compound **21-1**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (d, 1H, J = 8.4 Hz), 9.15 (s, 1H), 8.62 (s, 1H), 8.24 (s, 1H), 5.22 (s, 2H), 2.27 (s, 3H), 2.15 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.3, 147.0, 143.6, 141.5, 135.7, 121.9, 56.2, 19.9, 12.2.

21-2. *N*-(5-Hydroxy-3-(hydroxymethyl)-4,6-dimethylpyridin-2-yl)formamide (21). Triformyl compound **21-1** (5.62 g, 22.3 mmol) was dissolved in dry MeOH (25 mL) into which K_2CO_3 (1.54 g, 11.2 mmol) was added. This reaction mixture was stirred at 0 °C for 30 min. Citric acid (fine granule) was slowly added to the reaction mixture until the pH reached 7.0. The resulting slurry was filtered on Celite and the filter cake was completely washed with ice-cold MeOH. Filtrate was concentrated and purified on silica gel column chromatography (CH_2Cl_2 : MeOH = 15 : 1) to give **21** as a pale yellow solid in 68% for two steps. m.p. 118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.68 (d, 1H, J = 9.6 Hz), 8.92 (d, 1H, J = 10.5 Hz), 8.48 (s, 1H), 5.10 (t, 1H, J = 5.1 Hz), 4.48 (d, 2H, J = 5.4 Hz), 2.31 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.4, 147.0, 143.6, 141.6, 135.7, 121.9, 56.3, 19.9, 12.3; IR (KBr) ν 3437, 2923, 2848, 1666, 1455, 1377, 1250, 1111, 810, 790 cm^{-1}; MS (ESI+): *m/z* 197.0 $[M+H]^+$; HRMS: calcd for $C_9H_{12}N_2O_3$ $[M+Na]^+$ 219.0746, found 219.0739.

5-(Hydroxymethyl)-2,4-dimethyl-6-(methylamino)pyridin-3-ol (22). To a stirred solution of **21** (3.12 g, 15.9 mmol) in dry THF (150 mL) was added BH_3-THF complex (1.0 M in THF, 40.0 mL, 39.8 mmol). The reaction mixture was refluxed for 4 h and cooled down to room temperature. MeOH (10 mL) was slowly added and stirred for 30 min. The solvent was evaporated and the residue was purified over silica gel column chromatography (CH_2Cl_2 : MeOH = 4 : 1) to give **22** (2.55 g, 88%) as a light pink solid. m.p. 146–147 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.51 (s, 1H), 5.45 (br s, 1H), 4.85 (br s, 1H), 4.38 (s, 2H), 2.78 (s, 3H), 2.25 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 151.9, 141.6, 140.6, 135.4, 117.0, 56.5, 29.1, 19.9, 12.2; IR (KBr) ν 3362, 2923, 2853, 1743, 1636, 1458, 1412, 1381, 1228, 1153, 1092, 985, 709 cm^{-1}; MS (ESI+): *m/z* 138.1 $[M+H]^+$; HRMS: calcd for $C_9H_{14}N_2O_2$ $[M+H]^+$ 183.1128, found 183.1134.

2,2,5,7-Tetramethyl-2,4-dihydro-1*H*-pyrido[2,3-*d*][1,3]oxazin-6-ol (23a). A mixture of **20** (45 mg, 0.45 mmol), *p*-toluenesulfonic

acid monohydrate (8.6 mg, 0.04 mmol), 2,2-dimethoxypropane (6.0 mL) in dry DMSO (1.0 mL), THF (12 mL) and acetone (3 mL) was stirred at room temperature overnight. The reaction mixture was poured to sat. $NaHCO_3$ solution and organic layer was extracted with $CHCl_3$ (6 mL×3). The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, concentrated under high vacuum to give **23a** (66 mg, 70%). m.p. 171 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.64 (br s, 1H), 6.17 (s, 1H), 4.60 (s, 2H), 2.36 (s, 3H), 1.95 (s, 3H), 1.23 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.4, 142.8, 141.4, 131.5, 110.2, 81.9, 59.8, 26.7, 19.5, 11.0; IR (KBr) ν 3388, 2985, 2931, 2850, 1603, 1453, 1367, 1268, 1242, 1206, 1164, 1043, 805, 737 cm^{-1}; MS (ESI+): *m/z* 209.1 [M+H]$^+$; HRMS: calcd for $C_{11}H_{16}N_2O_2$ [M+H]$^+$ 209.1285, found 209.1281.

1,2,2,5,7-Pentamethyl-2,4-dihydro-1*H*-pyrido[2,3-*d*][1,3]oxazin-6-ol (23b). A mixture of **22** (230 mg, 1.26 mmol), *p*-toluenesulfonic acid monohydrate (48 mg, 0.25 mmol) and 2,2-dimethoxypropane (4.63 mL, 37.8 mmol) in dry DMSO (4.6 mL) was stirred at room temperature overnight. The reaction mixture was poured to sat. $NaHCO_3$ solution and organic layer was extracted with EtOAc (3 × 15 mL). The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified on a Et_3N treated silica gel column (hexanes : EtOAc = 3 : 2) to give **23b** (135 mg, 58%) as a caramel. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (s, 1H), 4.63 (s, 2H), 2.88 (s, 3H), 2.25 (s, 3H), 1.95 (s, 3H), 1.34 (s, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 146.8, 141.6, 141.2, 131.2, 112.0, 85.7, 59.7, 29.7, 23.8, 19.9, 11.1; IR (KBr) ν 3381, 2985, 2931, 1671, 1602, 1452, 1384, 1367, 1242, 1205, 1027, 1004, 804 cm^{-1}; MS (ESI+): *m/z* 223.1 [M+H]$^+$; HRMS: calcd for $C_{12}H_{18}N_2O_2$ [M+H]$^+$ 223.1441, found 223.1440.

2-Butyl-1,2,5,7-tetramethyl-2,4-dihydro-1*H*-pyrido[2,3-*d*][1,3]-oxazin-6-ol (23c). A mixture of **22** (436 mg, 2.39 mmol), camphorsulfonic acid (167 mg, 0.72 mmol) and 2-hexanone (8.84 mL, 71.7 mmol) in dry DMSO (3.5 mL) was stirred at room temperature overnight. The reaction mixture was poured to sat. $NaHCO_3$ solution and organic layer was extracted with EtOAc (3 × 15 mL). The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified on a Et_3N treated silica gel column (hexanes : EtOAc = 2 : 1) to give **23c** (278 mg, 44%) as a caramel. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.64 (s, 1H), 4.60 (s, 2H), 2.85 (s, 3H), 2.23 (s, 3H), 1.94 (s, 3H), 1.61–1.73 (m, 2H), 1.26–1.35 (m, 4H), 1.28 (s, 3H), 0.87 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR (75 MHz, CH_2Cl_2-d_2) δ 148.7, 142.3, 141.3, 131.7, 113.0, 88.6, 60.6, 37.3, 30.5, 25.8, 23.8, 22.3, 19.4, 14.7, 11.3; IR (KBr) ν 3333, 2956, 2928, 2856, 1600, 1460, 1405, 1244, 1220, 1058, 771, 740 cm^{-1}; MS (ESI+): *m/z* 265.2 [M+H]$^+$; HRMS: calcd for $C_{15}H_{24}N_2O_2$ [M+H]$^+$ 265.1911, found 265.1920.

5-(Hydroxymethyl)-2,4-dimethyl-6-(methyl(octyl)amino)pyridin-3-ol (24). To a solution of **22** (850 mg, 4.64 mmol) in MeOH (10 mL) were added 1-octanal (1.49 g, 11.6 mmol), acetic acid (0.88 mL, 15.5 mmol), sodium triacetoxyborohydride (1.08 g, 5.10 mmol). The reaction mixture was stirred for 1 h at room temperature. Solvent was removed under reduced pressure and the residue was poured to sat. $NaHCO_3$ solution. The organic layer was extracted with EtOAc (100 mL × 3), washed with water and brine and dried over anhydrous $MgSO_4$. After concentration, the residue was purified on silica gel column chromatography (hexanes : EtOAc = 4 : 1) to give **24** (995 mg, 73%) as a pale yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (s, 1H), 4.64 (s, 2H), 4.47 (dd, 1H, *J* = 4.5, 7.2 Hz), 3.30 (s, 2H), 2.85 (s, 3H), 2.24 (s, 3H), 1.93 (s, 3H), 1.63 (t, 2H, *J* = 5.1), 1.26–1.39 (m, 10H), 0.86 (t, 3H, *J* = 6.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.9, 142.1, 141.9, 131.2, 113.4, 87.6, 62.6, 32.6, 31.6, 31.0, 29.3, 29.1, 24.2, 22.5, 20.0, 14.4, 11.0; IR (KBr) ν 3309, 2925, 2855, 1595, 1467, 1407, 1323, 1234, 1121, 1033, 943, 769 cm^{-1}; MS (ESI+): *m/z* 295.2 [M+H]$^+$; HRMS: calcd for $C_{17}H_{30}N_2O_2$ [M+H]$^+$ 295.2380, found 295.2384.

13-HOTrE radical clock

Stock solutions of PFB-13-HOTrE (1.0 M), 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile) (MeO-AMVN) (0.05 M), and antioxidant (≈0.1 M) were prepared in benzene. Oxidation samples were prepared in 1 mL autosampler vials with a total volume of 100 µL. The solutions were added in the following specific order to prevent premature oxidation of the substrate: antioxidant (0.04–0.08 M), PFB-13-HOTrE (0.1 M), MeO-AMVN (2.5 mM), and diluted to 100 µL with benzene. The sealed vials were oxidized at 37 °C for 1 h. After 1 h, the reaction was stopped and the hydroperoxides were reduced by the addition of 50 µL of 0.1 M butylated hydroxytoluene (BHT) and $P(OMe)_3$ in hexanes. The samples were then prepared for LC-APCI-MS analysis. The reaction mixtures were loaded onto Varian Bond Elut, Jr. SPE cartridges that contained 500 mg of silica that had been pre-conditioned with 3 mL hexanes. After loading the cartridges, the samples were washed with 5 mL of a hexanes : EtOAc mixture (85 : 15) to remove excess antioxidant and unoxidized PFB-13-HOTrE. The diols were eluted from the cartridge with 2 mL EtOAc. The solvent was removed under a stream of N_2. The diols were redissolved in 250 µL MeOH : H_2O (80 : 20) for LC-MS analysis. For LC-APCI-MS, the diols were separated using RP-HPLC using a Supelco Discovery C-18 column (4.6 mm × 25 cm) and a MeOH : H_2O solvent gradient (70 : 30→90 : 10) between 5–45 min at 1 mL min^{-1}. The mass spectrometer was operated with an APCI source in negative ion mode. The instrumental conditions were as follows: 5 µA corona discharge, 300 °C capillary temperature, 475 °C vaporizer temperature, capillary voltage –20 V, and tube lens voltage –35 V. The diols were then analyzed using selective ion monitoring (SIM) of masses 309 and 291 *m/z*. The peak area ratio of the 8,13-*c*,*c*,*t* diols were then compared to the rest of the oxidation products after adjusting the peak areas by their respective response factors. The response factors convert the individual areas of the mass spectrometry peaks to match those of HPLC peak areas from controlled oxidations using α-TOH as the H-atom donor. A calibration curve from the α-TOH oxidations is then utilized to determine k_{inh}.

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Notes and references

1 (*a*) L. Valgimigli, G. Brigati, G. F. Pedulli, G. A. DiLabio, M. Mastragostino, C. Arbizzani and D. A. Pratt, *Chem.–Eur. J.*, 2003, **9**, 4997–5010; (*b*) M. Wijtmans, D. A. Pratt, L. Valgimigli, G. A. DiLabio, G. F. Pedulli and N. A. Porter, *Angew. Chem., Int. Ed.*, 2003, **42**, 4370–4373.

2 (*a*) M. Wijtmans, D. A. Pratt, J. Brinkhorst, R. Serwa, L. Valgimigli, G. F. Pedulli and N. A. Porter, *J. Org. Chem.*, 2004, **69**, 9215–9223; (*b*) T.-g. Nam, M. Wijtmans, D. A. Pratt and N. A. Porter, *Synthesis*, 2005, 1397–1404; (*c*) H.-Y. Kim, D. A. Pratt, J. R. Seal, M. Wijtmans and N. A. Porter, *J. Med. Chem.*, 2005, **48**, 6787–6789; (*d*) T.-g. Nam, C. L. Rector, H.-Y. Kim, A. F.-P. Sonnen, R. Meyer, W. M. Nau, J. Atkinson, J. Rintoul, D. A. Pratt and N. A. Porter, *J. Am. Chem. Soc.*, 2007, **129**, 10211–10219; (*e*) S. Kumar, H. Johansson, T. Kanda, L. Engman, T. Müller, M. Jonsson, G. F. Pedulli, S. Petrucci and L. Valgimigli, *Org. Lett.*, 2008, **10**, 4895–4898; (*f*) S. Kumar, H. Johansson, T. Kanda, L. Engman, T. Müller, H. Bergenudd, M. Jonsson, G. F. Pedulli, R. Amorati and L. Valgimigli, *J. Org. Chem.*, 2010, **75**, 716–725; (*g*) Y. Omata, Y. Saito, Y. Yoshida, B.-S. Jeong, R. Serwa, T.-g. Nam, N. A. Porter and E. Niki, *Free Radical Biol. Med.*, 2010, **48**, 1358–1365; (*h*) J. Lu, O. M. Khdour, J. S. Armstrong and S. M. Hecht, *Bioorg. Med. Chem.*, 2010, **18**, 7628–7638; (*i*) J. Lu, X. Cai and S. M. Hecht, *Org. Lett.*, 2010, **12**, 5189–5191; (*j*) R. Serwa, T.-g. Nam, L. Valgimigli, S. Culbertson, C. L. Rector, B.-S. Jeong, D. A. Pratt and N. A. Porter, *Chem.–Eur. J.*, 2010, **16**, 14106–14114; (*k*) T.-g. Nam, J.-M. Ku, H.-g. Park, N. A. Porter and B.-S. Jeong, *Org. Biomol. Chem.*, 2011, **9**, 1749–1755.

3 (*a*) G. W. Burton, T. Doba, E. J. Gabe, L. Hughes, F. L. Lee, L. Prasad and K. U. Ingold, *J. Am. Chem. Soc.*, 1985, **107**, 7053–7065; (*b*) G. W. Burton and K. U. Ingold, *Acc. Chem. Res.*, 1986, **19**, 194–201; (*c*) J. S. Wright, D. A. Pratt, G. A. DiLabio, T. P. Bender and K. U. Ingold, *Cancer Detect. Prev.*, 1998, **22**, 204; (*d*) M. C. Foti, E. R. Johnson, M. R. Vinqvist, J. S. Wright, L. R. C. Barclay and K. U. Ingold, *J. Org. Chem.*, 2002, **67**, 5190–5196.

4 H. J. Kayden and M. G. Traber, *J. Lipid Res.*, 1993, **34**, 343–358.

5 G. W. Burton and K. U. Ingold, *J. Am. Chem. Soc.*, 1981, **103**, 6472–6477.

6 C. L. Rector, D. F. Stec, A. R. Brash and N. A. Porter, *Chem. Res. Toxicol.*, 2007, **20**, 1582–1593.

7 B. Roscheck Jr., K. A. Tallman, C. L. Rector, J. G. Gillmore, D. A. Pratt, C. Punta and N. A. Porter, *J. Org. Chem.*, 2006, **71**, 3527–3532.

