Synthesis of a Potential Bifunctional Mimic of Transaminases

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As a potential bifunctional mimic of transaminases 3,7-dimethyl-10-[3-(4-aminomethyl-5-hydroxy-6-methyl-3-pyridyl)propyl]-3,7,10-triazatricyclo[3.3.0.0²⁵]undecane (I) has been synthesized by attaching 3,7-dimethyl-3,7,10-triazatricyclo[3.3.0.0²⁵]undecane (II) to a pyridoxamine nucleus via an all-carbon chain. The chain length between the pyridine ring and II is restricted to three atom units so that the possibility for II to act bifunctionally during the transamination is maximized. In its protonated form, the nitrogen closest to the pyridine ring cannot deliver the proton intramolecularly to the α-carbon of the developing amino acid. To make the synthetic route generally applicable, introduction of the side-arm base is arranged at a later stage of the synthesis so that different di- or poly-amine can easily be used in place of II to prepare other target molecules that might possess bifunctional catalytic activity. This arrangement also greatly reduces the polarity and water-solubility of the intermediates and the purification of these compounds thus becomes much easier. The method of introducing the amino functionality at the C-4 methylene group described herein provides an alternative to that currently in use (reduction of oximes).

Pyridoxamine is one of the B6 group of vitamins that plays an essential role in growth and maintenance of many life processes. As a model for the transaminase systems, pyridoxamine has been the subject of intensive studies of various kinds since the 1950s. Recent efforts in this field have been directed towards making model compounds which have the potential to reproduce certain features of natural transaminases. For instance, to accelerate the reaction several monoamines have been attached to the pyridoxamine nucleus via side-chains of different length. A still better mimic contains both a catalytic group (ethylenediamine) and a binding group (β-cyclodextrin).

The key step (the rate-limiting step under most circumstances) in the transamination is a 1,3-proton transfer process, with deprotonation taking place at one end of the aza-allyl system while reprotonation occurs at the other end (Fig. 1). If a polyamine of appropriate three-dimensional structure is present in the intermediate ketamine it might catalyze the 1,3-proton transfer process in a bifunctional way; while one nitrogen in the polyanine abstracts the proton from the C-4 methylene group (pyridoxamine numbering), the other nitrogen (in protonated form) delivers another proton to the α-carbon of the developing amino acid (Fig. 2).

Bifunctional catalysis in other 1,3-proton transfer reactions has been a research topic in our group for several years. According to a computer-assisted molecular-modeling study, mono- and di-protonated I possess great potential to catalyze 1,3-proton transfer in allyl systems in a bifunctional way (Fig. 3). To explore bifunctional catalysis

Fig. 1. The ketamine–aldimine tautomeration in the presence of Zn²⁺. X represents the remainder of the model compound.

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Fig. 2. A polyamine of appropriate three-dimensional structure in the ketimine-Zn$^{2+}$ complex might catalyze the 1,3-proton transfer in a bifunctional way; while one N abstracts a proton from C-4 methylene group, the other protonated N delivers a proton to the $\alpha$-carbon of the developing amino acid.

in the new context of imitating enzymes, we hoped that attachment of such a polyamine to the pyridoxamine nucleus would produce a new and efficient mimic of transaminases featuring bifunctional catalysis. In order to maximize the possibility for the base to act bifunctionally, the length of the chain between the pyridoxamine nucleus and 2 is restricted to three carbon units, so that the nitrogen atom abstracting the proton from the methylene group at C-4 cannot reach the $\alpha$-carbon of the developing amino acid.

**Results and discussion**

Because of the concatenation of the two moieties, pyridoxamine and 2, the target molecule 3 is expected to be a very polar, water-soluble, and air-sensitive substance. The choice of the precursor leading directly to the target molecule 3 is therefore of critical importance. An ideal precursor should be reasonably stable in air, have relatively low polarity and low solubility in water, while its conversion into 3 should not lead to extensive formation of side-products. Fig. 4 shows some of the compounds which are likely to meet these requirements. They all contain fewer basic nitrogen atoms than 3. The acidic phenolic hydroxy group is also masked.

The first precursor chosen in this work was compound 4. The corresponding synthetic route is shown in Scheme 1. Incorporation of the amine 2 is arranged at the final stage of the synthesis so that the polarity of all intermediates can be kept within the limits convenient for chromatography on silica gel. An additional advantage gained in doing this is that the same sequence may easily be adapted for the preparation of other target molecules by using other polyamines in place of the amine 2. The terminal vinyl group in the side arm serves as a protective group which is stable enough to withstand all the reaction conditions in the early stages of the sequence.

Protection of pyridoxine as acetonide (8) was performed as described previously. Subsequent oxidation with activated MnO$_2$ led to the aldehyde 9. Treatment of the aldehyde 9 with a slight excess of allyl Grignard reagent at $-70^\circ$C gave the alcohol 10 in 86% yield after chromatography, together with a small amount of the starting aldehyde. Deoxidation of the mesylate made *in situ* from 10 with lithium triethylborohydride provided 11 as a thick oil or a wax in ca. 90% yield.

Cleavage of the ketal 11 in aqueous MeOH yielded a highly water-soluble diol (12). Owing to the presence of the basic nitrogen atom in the pyridine ring, more than 1 equivalent of acid was necessary to ensure a practical reaction rate. Sulfuric acid was used here because it could easily be removed by reaction with an excess of calcium carbonate followed by filtration (the high solubility of 12 in water made it impossible to remove acid by washing the organic solution with an aqueous basic solution). After removal of water by repeated co-evaporation with ethanol, 12 was

**Fig. 3.** A modeling study indicates that mono- and di-protonated 1 have great potential to catalyze 1,3-proton transfer processes bifunctionally. Its nor-methyl analogue (2) was therefore chosen as the catalytic part of the target molecule (3) of this work.
obtained as a yellowish solid which could be used directly in the following step.

Oxidation of the diol 12 with activated MnO₂ followed by condensation with hydroxylamine gave the oxime 14. Treatment of the crude oxime in refluxing acetic anhydride afforded the nitrile 15. Oxidative cleavage of the carbon-carbon double bond with KMnO₄ proceeded smoothly under the phase-transfer conditions described by Lee et al.⁸ to afford the acid 16 in ca. 75% yield. The subsequent coupling with 2,⁹ however, failed to give any satisfactory results; the acetyl group was far too reactive under the reaction conditions.

A straightforward solution to the problem was to use a more stable protective group. A benzyl group was thus chosen as the substitute. This alternative corresponded to adopting the precursor 5 instead of 4. An additional modification made in this partially renewed route (Scheme 2) was that the benzyl group was introduced at an earlier stage instead of continuing from the acetate 15, as this would lead to more stable intermediates and thus facilitate the purification.

The silylation catalyzed by 4-dimethylaminopyridine (DMAP) gave exclusively the mono silyl ether 17. As the effort to purify this compound only led to unnecessary loss of the material, the crude product was directly used in the subsequent step. Benzylation of 17 in DMSO at room temperature gave compound 18 (in 48% overall yield from 11). Removal of the silyl protective group in 18 with tetrabutylammonium fluoride (Bu₄N⁺F⁻) proceeded rapidly at room temperature, to give the alcohol 19 as a white, crystalline substance in 95% yield after chromatography.

Modification of the C-4 methylene group was effected by
a sequence similar to that used in the first route. Oxidation of the alcohol 19 with pyridium chlorochromate (PCC) yielded the aldehyde 20 in 91% yield. Treatment of 19 with activated MnO₂ in refluxing CH₂Cl₂ also gave 20. Condensation of the aldehyde 20 with hydroxylamine in the presence of sodium acetate provided the aldoxime 21 which in turn was converted into the corresponding nitrile by being refluxed in acetic anhydride. In preparative runs, the crude aldehyde (20, obtained from PCC oxidation) and the crude oxime were directly used in the subsequent reaction without any purification. The overall yield from 19 to 22 was 83%.

In sharp contrast with the smooth reaction of 15 to 16, oxidative cleavage of the carbon–carbon double bond in 22 under the same conditions led to extensive formation of side-products. The original route was therefore modified...
once more (Scheme 3). A Lemieux-Johnson oxidation\(^1\) (OsO\(_4\)/NaIO\(_4\)) was performed to remove one carbon from \(22\) thus yielding the corresponding aldehyde, which was immediately reduced with NaBH\(_4\) without any purification. By this procedure the dark osmates from the Lemieux-Johnson oxidation could easily be removed with no significant loss of material. Crude \(24\), after simple work-up, was practically pure as seen in the \(^1\)H and \(^13\)C NMR spectra and could be used directly in the subsequent step.

Conversion of \(24\) into the corresponding mesylate \(25\) with MsCl proceeded smoothly at room temperature. The mesylate was in turn transformed into the bromide \(26\) by being refluxed with LiBr in dry acetone. Coupling of the bromide \(26\) with the amine \(2\) in refluxing acetonitrile produced the expected compound \(6\) as an oil in 70% yield after chromatography, with higher than 99.5% purity as determined by HPLC. Direct coupling of \(25\) with the amine \(2\) also afforded \(6\), but the reaction was much slower.

Reduction of \(6\) with LiAlH\(_4\) was much more complex than we had expected. All the products were highly polar compounds that could not satisfactorily be isolated and purified by column chromatography. To investigate the problem we used \(22\), a less polar and much more easily accessible compound, as a model compound to examine the reduction. It was then found that substantial amounts of side-products formed well before the starting nitrile was fully consumed, whereas prolonged reaction led to over-reduced products without the pyridine chromophore. We also tried several other reducing agents (e.g., BH\(_3\),\(^11\) NaBH\(_4\)/CoCl\(_2\)) but none of them gave satisfactory results. Although we did manage to obtain compound \(29\) by quenching the reduction at an early stage followed by acetylation with acetic anhydride, the low yield would make it extremely difficult to continue further if we applied the same procedure to the conversion of \(6\) into \(3\).

Rosen et al.\(^13\) recently reported a procedure which converted azides into acetamides under very mild conditions. Application of this method in the present case would result in an acetamido group at the C-4 methylene group. The reduction problem mentioned above could thus be avoided altogether (Scheme 4). The azide \(28\) was first prepared by treating \(19\) with methanesulfonyl chloride at room temperature followed by NaN\(_2\). Without using an excess of MsCl the first step (the reaction of \(19\) with MsCl) was rather

\[\text{Scheme 4. } a, \text{MsCl/NET}_3/\text{DMA/CH}_2\text{Cl}_2/\text{r.t. or MeLi/MeCl/THF/70°C}; b, \text{NaN}_2/\text{DMF}; c, \text{MeCOSH}; d, \text{O}_2/\text{MeOH/r.t.}; e, \text{NaBH}_4/\text{MeOH};\]
\[f, \text{OsO}_4/\text{NaIO}_4/\text{THF/H}_2\text{O}; g, \text{NaBH}_4/\text{MeOH/0°C}; h, \text{MsCl/NET}_3/\text{DMA/CH}_2\text{Cl}_2; i, \text{LiBr/acetone/reflux}; j, \text{NaHCO}_3/2/\text{MeCN};\]
\[k, 48% \text{ HB}{\text{r/reflux.}}\]
sluggish. The yield (crude mass of the intermediate) could vary considerably from run to run simply because of some minor difference in e.g., work-up, and was often much lower than the theoretical yield. Larger amounts of MsCl did not significantly improve the yield (in such cases, the intermediate isolated was identified as the chloride 27a). By using a smaller amount of MsCl and quenching the reaction at an early stage followed by flash column chromatography on silica gel, it was possible to obtain 27b as an unstable oil. This oil rapidly yielded a pink substance of very high polarity as shown by TLC. When allowed to stand at room temperature, it became a solid insoluble in common organic solvents.

The instability of the mesylate 27b convinced us of that the yield of 28 could be improved if the mesylate 27b was generated at a much lower temperature and treated directly with NaN₃ without a preceding work-up. Indeed, treatment of 19 in THF with 1 equiv. of MeLi followed by 1 equiv. of MsCl at −70°C resulted in a yellowish clear solution, in contrast with the dark-red mixture obtained in previous runs. Addition of NaN₃ and DMF (to increase the solubility of NaN₃) to the reaction system led to a yellowish suspension after a few minutes of stirring at room temperature and complete conversion into 28 was realized within two hours in 77% overall yield (from 19).

Subsequent transformation of the azide 28 into the aceta-
mide 29 by thioacetic acid proceeded very smoothly at room temperature. The reaction solution solidified near the end of the reaction. After removal of the excess of thioacetic acid, the crude product was chromatographed on silica gel to afford the pure acetamide 29 in 77% yield.

It is noteworthy that in the ¹H NMR spectrum of 29 (as well as all the other intermediates with the same C-4 CH₂ acetamido partial structure) the C-4 CH₂ appeared as a sharp two-line signal which could easily be mistaken as the two inner lines of an AB system with two outer lines buried in the noise. The other possibility that the line splitting was caused by coupling with the amide NH did not seem to be likely since the amide NH signal was very broad, with no recognizable splitting. When this broad NH peak was irradiated, however, the CH₂ doublet collapsed into a sharp singlet. Irradiation at the doublet, moreover, led to recognizably sharpening of the NH signal. Such a coupling relation was also unequivocally confirmed by a very strong cross peak in the COSY spectrum (2D NMR).

Up to this point the modification of the C-4 CH₂ position was complete. A free amino group could be easily generated without involvement of a reduct reaction. Before connecting the pyridoxamine moiety with the amine 2, the only remaining task was to activate the side chain by converting the terminal vinyl group into a bromide. Unexpectedly, the previous easy and clean conversion (OsO₄/NaIO₄ oxidative cleavage followed by NaN₃ reduction) turned out to be troublesome in this case; the intermediate aldehyde was highly polar and water-soluble. Neither aqueous work-up nor column chromatography could separate the intermediate aldehyde from the inorganic salts and osmates in acceptable yields. In the presence of these iodine-containing inorganic salts and osmates, reduction of the aldehyde with NaBH₄ led to complex products.

In the hope of finding a better method of preparing 30 we also attempted to modify the side chain before conversion of the azido group into the acetamido group. With an azido instead of an acetamido group at the C-4 CH₂ position, the aldehyde produced by OsO₄/NaIO₄ was indeed less water-soluble. Subsequent reduction with NaBH₄ followed by conversion of the azido group into an acetamido group with thioacetic acid gave 30 in improved yields. An even better yield was later obtained by ozonolysis of 29 followed by reductive work-up. Thus, treatment of a methanolic solution of 29 with ozone at room temperature (at low temperature 29 simply precipitated and no reaction with ozone took place) followed by reduction with NaBH₄ gave 30 as the only product.

Further transformation of 30 into the bromide 32 was accomplished in the same way, as for 24, except that aqueous work-up was avoided. Coupling of 32 with the amine 2 under the same conditions used for the preparation of 6 furnished the expected precursor 7 in 78% yield after column chromatography. Finally, the acetyl and the benzyl protective groups were removed by heating in 48% HBr to afford the final product 3 as a white, air-sensitive powder.

The ¹H NMR spectrum of 3 was strongly pH-dependent. At near neutral pH the spectrum was rather complicated, indicating that 3 existed in several forms (with different numbers of deuterons attached at the nitrogens). Simplifica-
tion of the spectrum was achieved at either strongly basic or strongly acidic pH.

The transamination activity of 3 was examined under the conditions described by Martell et al.¹⁴ and Breslow et al.² Surprisingly, at pH 4 the reaction was so slow that even after 24 h very little of the aldimine was produced in this reaction system. At higher pH (near pH 7), however, the transamination could be followed by UV-VIS spectro-
photometry. The spectra were similar to those of the compo-
unds with only one amine group in the side arm, except that the maximum absorption for the aldimine-Zn²⁺ appeared at 395 nm instead of 385 nm. The rate was still lower than that with pyridoxamine. The reasons for this behavior are under investigation.

Experimental

¹H and ¹³C NMR spectra were recorded on a Varian XL-400 NMR spectrometer (operating at 400 MHz for ¹H) with Me₄Si as an internal standard and CDCl₃, as the solvent unless otherwise specified. The figures in the parentheses following carbon chemical shifts are chemical shifts of the protons to which the carbons are directly bonded as seen in HETCOR spectra (2D-NMR). IR spectra were collected on a Perkin-Elmer 1600 FT infrared spectrometer. The letters in parentheses after wavenumber values refer to the relative intensity (s = strong, m = medium and w = weak).
Mass spectra (GC/MS) were obtained on a modified Finnigan Mat 1020B instrument (electron impact mode, 70 eV). The relative intensity of the peaks is given in parentheses after the corresponding m/z value. High resolution mass spectra (HRMS) were recorded at room temperature on a ZAB-HF machine operating in FAB mode (fast atom bombardment) with 3-nitrobenzyl alcohol as the solvent. Xe as the fast atom and polyethylene glycol as the reference for exact mass measurement. Melting points were determined on a Reichert KIFA micromelting point apparatus and are uncorrected. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden. Pyridoxine hydrochloride (98%), tert-butyldimethylsilyl chloride (TBDMS-Cl, 97%), LiBEt₄H (Superhydride, 1.0 M in THF), allylmagnesium chloride (2.0 M in THF), Bu₂N⁺F⁻ (1.0 M in THF), and Adogen 464 were purchased from Aldrich. Pyridinium chlorochromate (98%), MeLi (1.6 M in ether), activated MnO₂ (95%), and NaH (55–60% suspension in mineral oil) were purchased from FluKu AG. Silica gel for column chromatography (Kieselgel 60, 200–400 mesh) was purchased from Riedel-de Haën AG. Dry solvents and reagents were obtained in the following manner. THF was refluxed over Na/benzophenone and distilled under a nitrogen atmosphere prior to use. CH₂Cl₂ and NEt₃ were refluxed over CaH₂ and distilled under nitrogen prior to use. Dimethyl sulfoxide (DMSO) was refluxed over Na and distilled under a nitrogen atmosphere and stored over 4 Å molecular sieves. N,N-Dimethylformamide (DMF) was distilled over 4 Å molecular sieves. Diethyl ether was dried over Na wire and the supernatant was used directly in the work-up of 20. Acetone was kept over anhydrous K₂CO₃. LiBr was dried at 120°C/0.5 mmHg for 72 h. Aqueous NH₃ refers to 25% ammonia solution in water. Aqueous NH₄-saturated diethyl ether for column chromatography was prepared by shaking 25% aqueous NH₃ with diethyl ether and separating the etheral phase.

2,2,8-Trimethyl-5-(3-hydroxy-3-butenyl)-4H-1,3-dioxino [4,5-c]pyridine (10). Allylmagnesium chloride (about 2.3 M, 8.5 ml) was added dropwise via a syringe to a stirred solution of the aldehyde 9 (4.00 g, 19.3 mmol) in dry THF (40 ml) at −70°C (bath temperature) under N₂. After a further 20 min of stirring, the bath temperature was allowed to rise to room temperature and the reaction was quenched with aq. NH₄Cl. The reaction mixture was diluted with ether, washed once with brine and dried over MgSO₄. Filtration and evaporation left a white solid (4.69 g), which was chromatographed on silica gel (aq. NH₄-saturated diethyl ether) to afford 4.16 g (86%) of pure 10; m.p. 80–81°C. 1H NMR: δ 7.98 (1H, s), 5.80 (1H, m), 5.20–5.15 (2H, m), 4.93 (2H, s), 4.66 (1H, m), 2.69 (1H, br s, OH), 2.52 (2H, m), 2.38 (3H, s), 2.15 (3H, s), 1.35 (3H, s), 1.53 (3H, s). 13C NMR: δ 147.36, 145.80, 137.68 (7.98), 133.78 (5.80), 131.90, 124.43, 119.16 (5.20–5.15), 99.42, 69.26 (4.66), 58.72 (4.93), 42.13 (2.52), 24.95 (1.55), 24.55 (1.53), 18.50 (2.34). IR(KBr): 3166 (s), 1643 (w), 1602 (w), 1567 (w), 1402 (s), 1137 (s), 1055 (s), 850 (m) cm⁻¹. MS m/z: 249 (M⁺, 28), 208 (32), 191 (17), 173 (36), 162 (71), 150 (83), 122 (100). Anal. C₁₀H₁₂NO₅; C, H, N.
ature then for 35 min at 50 °C, and finally cooled at 0 °C, to yield a beige powder which was collected by suction filtration, washed with cold water, and dried at 50 °C. The resulting crude 14 (720 mg, 71 %) was then refluxed in acetic anhydride (15 ml) under N2 for 1.5 h. The excess acetic anhydride was removed in vacuo and the residue was chromatographed on silica gel (1.5:1 hexane–ethyl acetate), giving a white solid (15, 694 mg, 52 % from 11); m.p. 58–60 °C. 1H NMR: δ 8.42 (1 H, s), 5.82 (1 H, m), 5.07–5.01 (2 H, m), 2.91 (2 H, t, J 7.6 Hz), 2.49–2.41 (8 H, m, including a singlet at 2.45, 2 × CH3 and 1 × CH2). 13C NMR: δ 167.71, 150.81, 147.52 (8.42), 145.69, 137.67, 135.82 (5.82), 116.78 (5.07–5.01), 115.56, 112.63, 34.34 (2.49–2.41), 30.91 (2.91), 20.50 (2.49–2.41), 19.21 (2.49–2.41). IR (KBr): 2226 (w), 1766 (s), 1637 (w), 1590 (w), 1379 (m), 1167 (s), 914 (m) cm⁻¹. MS m/z: 230 (M⁺, 4), 188 (17), 147 (12), 119 (6), 43 (100). Anal. C₁₂H₁₀N₂O₄ C, H, N.

An analytical sample of 12 was obtained by column chromatography on silica gel (100:10:1 ethyl acetate–MeOH–aq. NH₃); m.p. 100–105 °C. 1H NMR: δ 7.85 (1 H, br s, OH), 7.62 (1 H, s), 5.75 (1 H, m), 5.05 to 4.95 (5 H, m, including 4.97 s and an OH), 2.54 (2 H, br t, J 8.0 Hz), 2.38 (3 H, s), 2.18 (2 H, m). 13C NMR: δ 152.42, 144.75, 138.05 (7.62), 136.81 (5.75), 132.12, 130.74, 115.75 (5.05 to 4.95), 60.21 (4.97), 34.51 (2.18), 29.05 (2.54), 17.69 (2.38). IR (KBr): 3061 (s, br), 2602 (s), 1637 (w), 1555 (w), 1414 (s), 1285 (s), 909 (s), 750 (m) cm⁻¹. MS m/z: 193 (M⁺, 91), 175 (36), 160 (66), 152 (47), 106 (100). Anal. C₁₂H₁₀N₂O₄ C, H, N.

Similarly, an analytical sample of 14 was obtained by column chromatography on silica gel (aq. NH₃-saturated diethyl ether); m.p. 183–184 °C. 1H NMR: δ 10.35 (1 H, br s, OH), 9.74 (1 H, br s, OH), 8.51 (1 H, s), 7.89 (1 H, s), 5.84 (1 H, m), 5.10–5.03 (2 H, m), 2.79 (2 H, br t, J 7.8 Hz), 2.51 (3 H, s), 2.33 (2 H, m). 13C NMR (CD₂OD): δ 152.21, 148.34 (8.51), 146.67, 140.27 (7.89), 138.08 (5.84), 134.61, 121.77, 116.30 (5.10–5.03), 36.21 (2.33), 29.93 (2.79), 18.22 (2.51). IR (KBr): 3400–2026 (m, br), 1766 (m, br), 1637 (m), 1484 (s), 1396 (s), 1238 (s), 1038 (m), 914 (m), 726 (m) cm⁻¹. MS m/z: 206 (M⁺, 12), 189 (11), 188 (13), 178 (13), 165 (18), 148 (100), 119 (70), 92 (28). Anal. C₁₂H₁₀N₂O₄ C, H, N.

3-(5-Acetoxy-4-cyano-6-methyl-3-pyridyl)propionic acid (16). Solid KMN₈O₄ (507 mg, 3.2 mmol) was added (in small portions over 20 min) to a stirred two-phase mixture of Adogen 464 (22 mg), glacial acetic acid (130 μl, 2.3 mmol), 15 (1.13 mmol), aq. H₂SO₄ (0.96 M, 17.1 ml) and CH₃Cl₂ (6.5 ml) at 0°C. Stirring was then continued at room temperature for a further 100 min before the reaction mixture was suction-filtered. The filter cake was washed with CH₃Cl₂ and H₂O. The brown filtrate and washings were extracted three times with CH₃Cl₂ and all CH₃Cl₂ phases were combined and dried over Na₂SO₄. Filtration and concentration in vacuo left ca. 4 ml of residue which crystal-
From a small sample of purified 17 (silica gel, 100:4:1 ethyl acetate–MeOH–aq. NH₄OH) were obtained the following data: m.p. 71–71.5 °C. ¹H NMR δ 8.82 (1 H, s, OH), 7.82 (1 H, s), 5.82 (1 H, m), 5.08–4.99 (2 H, m), 4.97 (2 H, s), 2.56 (2 H, br t, J 7.8 Hz), 2.44 (3 H, s), 2.25 (2 H, m), 0.96 (9 H, s), 0.19 (6 H, s). ¹³C NMR δ 150.88, 145.67, 140.24 (7.82), 136.93 (5.82), 130.70, 127.61, 115.60 (5.08–4.99), 62.12 (4.97), 34.85 (2.25), 29.14 (2.56), 25.66 (0.96), 18.74 (2.44), 18.11, –5.59 (0.19). IR (KBr): 3676–2000 (m, v br), 2552 (m, br), 1642 (w), 1560 (w), 1414 (m), 1223 (s), 1070 (s), 840 (s), 776 (s) cm⁻¹. MS m/z: 307 (M⁺, 11), 250 (100), 232 (52), 176 (32), 160 (14), 75 (87). Anal. C₃H₇NO₅Si: C, H, N.

3-Benzyl-5-(3-butenyl)-2-methyl-4-pyridinecarboxaldehyde (20). To a stirred mixture of alcohol 19 (501 mg, 1.77 mmol) and anhydrous NaOAc (440 mg) in dry CH₂Cl₂ (13 ml) under nitrogen was added pyridinium chlorochromate (776 mg, 3.53 mmol). Stirring was continued at room temperature for 3 h before the reaction mixture was filtered through a short pad of silica gel (eluted with dry ether) to remove the colored chromium species. The filtrate was concentrated to minimum volume and chromatographed on silica gel (3:1 hexane–ethyl acetate) to afford a yellowish oil, which solidified with time. 20 (457 mg, 92 %; m.p. 31–31.5 °C. ¹H NMR: δ 10.39 (1 H, s), 8.26 (1 H, s), 7.43–7.34 (5 H, m), 5.83 (1 H, m), 5.04–4.96 (4 H, m, containing 4.97 s), 2.95 (2 H, t, J 7.6 Hz), 2.59 (3 H, s), 2.29 (2 H, m). ¹³C NMR: δ 192.12 (10.39), 154.57, 152.73, 147.37 (8.26), 137.39 (5.83), 135.31, 134.71, 133.14, 128.97 (7.43–7.34), 128.87 (7.43–7.34), 128.55 (7.43–7.34), 115.61 (5.04–4.96), 77.92 (4.97), 35.24 (2.29), 29.50 (2.95), 19.23 (2.59). IR (KBr): 1692 (s), 1637 (w), 1540 (w), 1447 (m), 1355 (s), 1240 (s), 1186 (s), 908 (s), 690 (s) cm⁻¹. MS m/z: 281 (M⁺, 5), 263 (2), 189 (2), 162 (3), 91 (100). Anal. C₁₈H₁₃NO₅: C, H, N.

3-Benzyl-5-(3-butenyl)-2-methyl-4-pyridinecarbonitrile (22). A mixture of NaOAc (630 mg), NH₄OH·HCl (317 mg), and the crude aldehyde (20, prepared from 612 mg of 19 by PCC oxidation) inaq. MeOH (1 ml of MeOH plus 3 ml of water) was stirred at room temperature for 10 min, at 50°C for another 10 min, and finally cooled to 0°C for 10 min. The yellowish solid was collected by suction filtration, washed with cold water, and dried under an IR heating lamp. The off-white powder (crude 21, 570 mg, 1.92 mmol) was stirred in acetic anhydride (4 ml) and heated under reflux for 1.5 h. The excess acetic anhydride was removed in vacuo. MeOH was added to the residue and the resultant solution was evaporated to the minimum volume on a rotary evaporator. Chromatography of the residue on silica gel (3:1 hexane–ethyl acetate) gave a white solid (22, 498 mg, 1.79 mmol, 83 % from 19); m.p. 54.5–55.5 °C. ¹H NMR: δ 8.25 (1 H, s), 7.54–7.37 (5 H, m), 5.83 (1 H, m), 5.17 (2 H, s), 5.06–5.00 (2 H, m), 2.90 (2 H, t, J 7.5 Hz), 2.48 (3 H, s), 2.44 (2 H, m). ¹³C NMR: δ 153.64, 151.98, 144.82 (8.25), 137.69, 136.16 (5.83), 135.40, 128.91 (7.50–7.37), 128.75 (7.50–7.37), 128.73 (7.50–7.37), 116.61 (5.06–5.00), 114.19, 114.05, 76.59 (5.17), 34.49 (2.44), 30.93 (2.90), 19.45 (2.48). IR (KBr): 2231 (w), 1644 (w), 1583 (w), 1545 (w), 1473 (m), 1410 (m), 1364 (s), 1194 (s), 914 (m), 746 (s) cm⁻¹. MS m/z: 278 (M⁺, 7), 91 (100). Anal. C₁₈H₁₃NO₂: C, H, N.

An analytical sample of 21 was obtained by recrystallization from methanol; m.p. 171–172 °C. ¹H NMR: δ 8.39 (1 H, s), 8.23 (1 H, br s, OH), 8.17 (1 H, s), 7.43–7.33 (5 H, m), 5.81 (1 H, m), 5.03–4.94 (2 H, m), 4.83 (2 H, s), 2.89 (2 H, t, J 7.7 Hz), 2.50 (3 H, s), 2.30 (2 H, m). ¹³C NMR: δ (5:2 of CD₂OD–DMSO-d₆) δ 152.42, 151.51, 146.34 (8.17), 144.82 (8.39), 138.59 (5.81), 137.47, 135.40, 133.78, 129.31 (7.43–7.33), 129.25 (7.43–7.33), 129.17 (7.43–7.33), 115.56 (5.03–4.94), 76.88 (4.83), 35.44 (2.30), 31.08 (2.89), 18.99 (2.50). IR (KBr): 2721 (m, v br), 1638 (w), 1586 (m), 1492 (m), 1361 (s), 994 (s), 900 (s), 743 (s) cm⁻¹; MS m/z: 296 (M⁺, 34), 278 (5), 205 (8), 191 (8), 147 (9), 91 (100). Anal. C₁₈H₁₃NO₂: C, H, N.

3-(5-Benzyl-4-cyano-6-methyl-3-pyridyl)propanol (24). To a stirred mixture of 22 (263 mg, 0.94 mmol), OsO₄ (6.4 mg, THF) (15 ml), and water (5 ml) was added solid NaOAc (1.4 g, 6.5 mmol, in portions over 80 min). Stirring was continued for another 2 h before the reaction mixture was suction-filtered. The filtrate was diluted with diethyl ether, washed once with water, twice withaq. NaHSO₄, and once withbrine. The combined aqueous phases were back-extracted once more with diethyl ether and washed as before. The etheral phases were combined and dried over Na₂SO₄. After removal of solvent, the residue [the intermediate aldehyde; ¹H NMR: δ 9.82 (1 H, s), 8.30 (1 H, s), 7.48–7.37 (5 H, m), 5.16 (2 H, s), 3.10 (2 H, t, J 7.4 Hz), 2.89 (2 H, t, J 7.4 Hz), 2.48 (3 H, s)] was dissolved in MeOH (10 ml) and treated with an excess of NaBH₄ at 0°C. The reaction was quenched with water and the product was taken up into diethyl ether. The etheral phase
was washed twice with water and dried over MgSO₄. The reddish residue obtained after removal of solvent was redissolved in diethyl ether and another portion of MgSO₄ was added to remove the colored species. Filtration and evaporation left a yellowish oil, which solidified with time (24, 217 mg); m.p. 80.5–82.5 °C. This crude product contained only negligible amounts of impurities (as shown by both ¹H and ¹³C NMR spectroscopy) and could be used directly for the following step. The overall yield was 82% (from 22).

Chromatography on silica gel (100:20:1 ethyl acetate–MeOH–aq. NH₄OH) gave an analytical sample; m.p. 81.5–82 °C. ¹H NMR: δ 8.28 (1 H, s), 7.50–7.38 (5 H, m), 5.16 (2 H, s), 3.70 (2 H, t, J 5.9 Hz), 2.90 (2 H, t, J 7.8 Hz), 2.47 (3 H, s), 1.93 (2 H, m), 1.82 (1 H, br s, OH). ¹³C NMR: δ 154.31, 152.59, 145.27 (8.28), 138.65, 135.94, 129.49 (7.50–7.38), 129.33 (7.50–7.38), 129.28 (7.50–7.38), 114.64, 77.16 (5.16), 62.04 (3.70), 33.70 (1.93), 28.35 (2.90), 19.96 (2.47). IR (KBr): 3297 (s, br), 2229 (w), 1581 (w), 1542 (w), 1497 (w), 1450 (m), 1408 (s), 1361 (s), 1214 (s), 1057 (s), 936 (m), 889 (m), 743 (s), 690 (s) cm⁻¹. MS m/z: 282 (M⁺, 7.9), 91 (100). Anal. C₁₇H₁₄BrN₂O₄: C, H, N.

3-(5-Benzoyloxy-4-cyano-6-methyl-3-pyridyl)propyl methanesulphonate (25). A solution of 24 (217 mg, 0.77 mmol), NET₃ (0.8 ml), DMAP (8 mg), and MsCl (65 µl) in CH₂Cl₂ (10 ml) was stirred at room temperature for 50 min before a further 15 µl of MsCl was introduced. Stirring was continued for another hour. The mixture was diluted with diethyl ether, washed twice with water and once with brine and dried over MgSO₄. Filtration and evaporation left a yellowish oil, which solidified when allowed to stand overnight at room temperature (276 mg, 0.76 mmol, 99%, m.p. 61–62.5 °C). An analytical sample was obtained by chromatography on silica gel (100:4:1 ethyl acetate–MeOH–aq. NH₄OH); m.p. 63–64 °C. ¹H NMR: δ 8.29 (1 H, s), 7.50–7.38 (5 H, m), 5.19 (2 H, s), 4.28 (2 H, t, J 6.1 Hz), 3.06 (3 H, s), 2.95 (2 H, t, J 7.7 Hz), 2.49 (3 H, s), 2.15 (2 H, m). ¹³C NMR: δ 153.75, 152.65, 144.44 (8.29), 136.27, 135.21, 128.88 (7.50–7.38), 128.70 (7.50–7.38), 128.63 (7.50–7.38), 113.93, 113.85, 76.62 (5.19), 67.92 (4.28), 37.58 (3.06), 29.77 (2.15), 27.49 (2.95), 19.54 (2.49). IR (KBr): 2224 (w), 1464 (w), 1404 (w), 1338 (s), 1164 (s), 979 (s), 815 (m), 701 (m) cm⁻¹. MS m/z: 360 (M⁺, 1), 149 (2), 91 (100). Anal. C₂₀H₁₇BrN₂O₄: C, H, N.

4-Azidoethyl-3-benzoyloxy-5-(3-butenyl)-2-methylpyridine (28): two-step procedure. An excess of MsCl (ca. 5 equivs., at ca. 2 h intervals, monitored by TLC) was added in portions via a syringe to a stirred mixture of 19 (440 mg, 1.55 mmol), DMAP (11 mg, 0.09 mmol), NET₃ (9.5 ml, 68 mmol) in dry CH₂Cl₂ (40 ml) at room temperature under N₂. When TLC showed a complete conversion, the reaction mixture was diluted with diethyl ether, washed with water and brine, and dried over Na₂SO₄. Filtration and evaporation gave crude 27 as a brown oil. To this oily residue were added NaN₃ (550 mg, 8.5 mmol) and dry DMF (5 ml). After being stirred for 15 min at 70 °C (hot-plate temperature) and 1 h at room temperature, the reaction mixture was diluted with ether, washed with water (3×4 ml) and brine (2×2 ml), and dried over Na₂SO₄. Filtration and evaporation gave crude 28 as a brown oil, which was chromatographed on silica gel (2.5:1 ethyl acetate–hexane) to furnish pure 28 (320 mg) as a yellowish oil with an overall
Chromatography on silica gel (3:1 ethyl acetate–hexane) gave pure 27a as a yellowish oil, which solidified when allowed to stand at −20 °C overnight; m.p. 46–46.5 °C.

4-Azidomethyl-3-benzoxo-5-(3-butenyl)-2-methylpyridine (28): one-pot procedure. MeLi (1.6 M, 240 μL, 0.38 mmol) was added dropwise via a syringe to a solution of 19 (100 mg, 0.35 mmol) in dry THF (5 mL) stirred at −70 °C under N2. After 5 min, MsCl (29 μL, 0.37 mmol) introduced via a syringe. Stirring was continued for 30 min during which the bath temperature was allowed to rise to 30 °C. To the reaction mixture was then added NaN3 (222 mg, 3.4 mmol), followed by dry DMF (5 mL). Stirring was continued for another 2 h. The reaction mixture was diluted with diethyl ether, washed with water and brine (twice each) and dried over Na2SO4. The crude oil after removal of solvent was chromatographed on silica gel (3:1 ethyl acetate–hexane) to afford pure 28 as an almost colorless oil (82 mg, 77% from 19).

-3-[4-Acetamidomethyl-5-benzoxo-6-methyl-3-pyridyl]-propanol (30) from 29 by ozonolysis. Ozone-containing air was bubbled into a methanolic solution of 29 (90 mg, 0.28 mmol, in 2 mL) at room temperature until TLC showed complete consumption of the starting material. After expulsion of the excess ozone in the solution with N2, NaBH4 (34 mg, 0.9 mmol) was added with cooling (ice-water bath) and stirring to the reaction mixture in small portions over 20 min. Stirring was continued for 30 min at room temperature before solid NH4Cl (104 mg) was introduced. After the addition, stirring was continued for another hour. The reaction mixture was evaporated to dryness on a rotary evaporator and then further evaporated with an oil pump. The white solid residue was triturated with CH2Cl2, filtered, and washed with more CH2Cl2. The combined CH2Cl2 filtrate and washings were evaporated to dryness to afford 86 mg of crude product. Chromatography on silica gel (10:3:0.5 ethyl acetate–MeOH–aq. NH3) gave pure 30 as a white solid (62 mg, 68%); m.p. 142–143 °C.

4-Azidomethyl-3-benzoxo-5-(3-butenyl)-2-methylpyridine (29). A mixture of 28 (227 mg, 0.74 mmol) in thioacetic acid (500 μL) was stirred at room temperature under N2 for 2.5 h. The excess thioacetic acid was removed in vacuo with occasional slight heating (50 °C hotplate). The residual yellowish solid was then chromatographed twice on silica gel (5:1.5 CH2Cl2–MeOH–hexane and 100:4 ethyl acetate–MeOH) to afford 184 mg of 29 (77%) as a white solid; m.p. 127–128 °C. 1H NMR: δ 8.18 (1 H, s), 7.43–7.39 (5 H, m), 5.80 (1 H, m), 5.62 (1 H, br s, NH), 5.40–4.96 (2 H, m), 4.89 (1 H, s), 4.35 (2 H, d, J 5.7 Hz), 2.76 (2 H, t, J 7.7 Hz), 2.57 (3 H, s), 2.28 (2 H, m), 1.84 (3 H, s). 13C NMR: δ 169.45, 152.09, 150.29, 146.05 (8.14), 137.77, 137.07 (5.80), 136.30, 134.79, 128.89 (7.43–7.39), 127.89 (7.43–7.39), 115.84 (5.40–4.96), 75.63 (4.89), 35.32 (2.28), 34.87 (4.35), 29.24 (2.76), 23.04 (1.84), 19.66 (2.57). IR (KBr): 3284 (s), 1637 (s), 1537 (s), 1214 (m), 691 (m) cm−1. MS m/z: 324 (M+, 0.8), 281 (0.7), 251 (0.4), 252 (0.3), 233 (0.6), 174 (0.6), 106 (6.4), 91 (100). Anal. C18H14N2O2: C, H, N.
were combined, diluted with CH₂Cl₂, washed with brine and aq. NaHCO₃ (once each) and dried over Na₂SO₄/MgSO₄. The crude oil after removal of solvent was immediately dissolved in MeOH (4 ml) and treated with an excess of NaBH₄ (112 mg, added in portions) at 0°C for 45 min before being diluted with CH₂Cl₂, washed with aqueous NH₄Cl, and dried over Na₂SO₄/MgSO₄. Filtration and evaporation left a red-brown oil (327 mg), which was immediately treated with triacontioic acid (2 ml) as described for the preparation of 29. The excess triacontic acid was removed in vacuo and the dark oily residue was chromatographed on silica gel (4.5:1:4.5, then 10:3:10, CH₂Cl₂-MeOH-hexane) to give 163 mg of 30.

3-[4-Acetamidomethyl-5-benzoyloxy-6-methyl-3-pyridyl]propyl methanesulphonate (31). The reaction conditions were the same as for the preparation of 25 from 24. When TLC showed complete conversion, the reaction mixture was concentrated to ca. half volume on a rotary evaporator and then chromatographed on silica gel (eluting with 5:3:5 CH₂Cl₂-MeOH-hexane) to furnish 31 as a white solid (93%); m.p. 128–128.5°C. ¹H NMR δ 8.14 (1 H, s), 7.46–7.40 (5 H, m), 5.78 (1 H, br s, NH), 4.91 (2 H, s), 4.36 (2 H, d, J 5.8 Hz), 4.25 (2 H, t, J 6.2 Hz), 3.05 (3 H, s), 2.80 (2 H, t, J 7.7 Hz), 2.57 (3 H, s), 1.99 (2 H, m), 1.83 (3 H, s). ¹³C NMR: δ 169.59, 152.35, 150.92, 145.83 (8.14), 138.12, 136.27, 133.64, 128.99 (7.46–7.40), 128.93 (7.46–7.40), 128.46 (7.46–7.40), 75.71 (4.91), 68.83 (4.25), 37.25 (4.05), 34.70 (4.36), 30.59 (1.99), 25.77 (2.80), 23.09 (1.83), 19.75 (2.57). IR (KBr): 3292 (m), 1643 (m), 1349 (s), 1175 (m), 984 (m), 842 (m) cm⁻¹. MS m/z: (M⁺, 0.4), 363 (1), 311 (1), 273 (2), 177 (2), 160 (3), 91 (100).


3-[4-Acetamidomethyl-5-benzoyloxy-6-methyl-3-pyridyl]propyl bromide (32). A mixture of 31 (111 mg, 0.27 mmol) and dry LiBr (235 mg, 2.7 mmol) in dry acetone (5 ml) under N₂ was heated to reflux with stirring for 25 min. After cooling to room temperature, the solids were removed by filtration. The greenish filtrate was concentrated in vacuo and chromatographed on silica gel (5:1 ethyl acetate-MeOH) to afford 32 as a white solid, which gave the following after being washed with hexane (to remove all volatile products from acetone) and drying (75 mg, 70%); m.p. 127–127.5°C. ¹H NMR: δ 8.17 (1 H, s), 7.46–7.38 (5 H, m), 5.66 (1 H, br s, NH), 4.91 (2 H, s), 4.35 (2 H, d, J 5.6 Hz), 3.42 (2 H, t, J 6.6 Hz), 2.83 (2 H, t, J 7.7 Hz), 2.58 (3 H, s), 2.09 (2 H, m), 1.85 (3 H, s). ¹³C NMR: δ 169.39, 152.20, 150.75, 146.02 (8.17), 137.92, 136.18, 133.62, 128.92 (7.46–7.38), 128.87 (7.46–7.38), 128.41 (7.46–7.38), 75.62 (4.91), 34.80 (4.35), 34.05 (2.09), 32.62 (3.42), 28.25 (2.83), 23.13 (1.85), 19.79 (2.58). IR (KBr): 3292 (m), 1649 (s), 1567 (m), 1371 (m), 1213 (m), 1066 (w), 755 (m), 706 (m) cm⁻¹. MS m/z: 392 (M⁺, 1), 390 (M⁺, 1), 349 (1), 347 (1), 311 (2), 259 (2), 257 (2), 243 (1), 241 (1), 177 (1), 160 (4), 148 (2), 132 (2), 121 (2), 106 (6), 92 (100). Anal. Cal. C₁₉H₂₃BrN₂O₃: C, H, N.

3,7-Dimethyl-10-[3-(4-acetamidomethyl-5-benzoyloxy-6-methyl-3-pyridyl)propyl]-3,7,10-triazatricyclo[3.3.3.0³⁷]undecane (7). A mixture of 32 (86 mg, 0.22 mmol), 2 (40 mg, 0.22 mmol) and NaHCO₃ (220 mg, 22 mmol) in dry acetonitrile (6 ml) under N₂ was heated to reflux with stirring for 14 h. The solids were filtered off and washed with MeOH. The yellowish filtrate and washings (ca. 8 ml) were concentrated on a rotary evaporator and applied to a silica gel column which had already been pre-eluted with MeOH-aq. NH₄OH (80:1) until the effluent was clear. Elution with the same solvents (80:1, then 40:1) under slightly positive pressure gave 7 as a clear oil (84 mg, 78%). ¹H NMR: δ 8.12 (1 H, s, pyridine H), 7.42–7.40 (5 H, m, Ph H), 5.93 (1 H, br s, NH), 4.88 (2 H, s, PhCH₂), 4.38 (2 H, d, J 5.2 Hz, AcNCH₂), 2.71 (2 H, t, J 7.3 Hz, the CH₂ adjacent to pyridine), 2.54 (3 H, s, pyridine CH₃), 2.53–2.46 (12 H) including 2.50 and 2.48 AB system (J 9.0 Hz, 4×CH₂ adjacent to NMe) and 2.48 [s, chain-N(CH₃)₂ in amine 2 moiety, 2.40 (t, J 7.1 Hz, 2 H, the CH₂ adjacent to amine 2 moiety), 2.31 (6 H, s, 2×NCH₃), 1.85 (3 H, s, CH₂CO), 1.68 (2 H, br quintet, the middle CH₂ in the side chain). ¹³C NMR δ 169.79, 152.56, 150.52, 147.78 (8.12), 138.16, 136.75, 135.81, 129.32 (7.42–7.40), 129.20 (7.42–7.40), 128.77 (7.42–7.40), 76.07 (4.88), 66.98 (2.50 and 2.48), 64.37 (2.48), 62.24, 53.72 (2.40), 41.97 (2.31), 35.40 (4.38), 30.44 (1.68), 27.74 (2.71), 23.61 (1.85), 20.15 (2.54).
IR (neat): 3270 (m), 2932 (s), 2790 (s), 1654 (s), 1540 (s), 1469 (s), 1365 (s), 1267 (s), 1112 (s), 733 (s) cm⁻¹. HRMS: Found, 492.337. Calc. for C₁₉H₂₃BrN₂O₃ (M⁺+1), 492.334.

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