A HETERO DIELS–ALDER ACCESS TO (*Z*)-ZEATIN AND (*Z*)-ISOZEATIN

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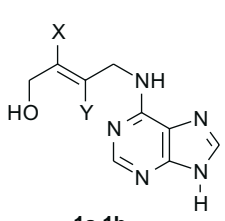
(*Z*)-6-(4-Hydroxy-3-methylbut-2-en-1-ylamino)purine, (*Z*)-zeatin, and the isomeric (*Z*)-isozeatin, both free from the *E* isomers, were prepared using the cycloaddition of the *in situ* generated *tert*-butyl nitrosoformate on isoprene. A mixture of *tert*-butyl 5(and 4)-methyl-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylates thus formed was deprotected and reductively cleaved to (*Z*)-4-amino-2(and 3)-methylbut-2-en-1-ols, which were chromatographically separated and finally alkylated with 6-chloropurine to give the title compounds.
Key words: Cytokinins; Zeatin; Nitrosoformates; Diene synthesis; 3,4-Dihydrooxazines; Cycloadditions; Diels–Alder reaction; Purines.

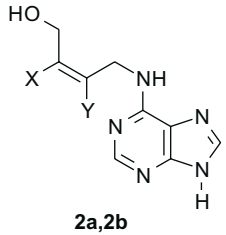
Both *E* and *Z* isomers of zeatin, 6-[4-hydroxy-3-methylbut-2-en-1-yl)-amino]purine are important members of the large family of natural cytokinins. While the chemistry of (*E*)-zeatin (**1a**) has been thoroughly elaborated since its isolation by Letham and coworkers in 1964 (ref.[1]) and the compound is synthetically available by several methods[2,3], (*Z*)-zeatin (**2a**) is accessible only with difficulties. Compounds **1a** and **2a** are prepared by condensation of 6-chloropurine with aminoalcohol **3a** and **4a**, respectively. The main problem associated with the synthesis of **2a** is the lack of a simple preparative pathway for **4a**. So far, four methods, differing in reliability and/or practical applicability, have been suggested for the purpose.

A stereoselective access to **4a**, based on the reductive cleavage of the N–O bond in 5-methyl-3,6-dihydro-2*H*-1,2-oxazine (**7a**) appeared as early as 1971. In this paper[4], the dihydrooxazine was prepared by a Diels–Alder reaction of 1-chloro-1-nitrosocyclohexane and isoprene, followed by alcoholysis of the primary adduct, in the same way as described for the cycloaddition of 1-chloro-1-nitrosocyclohexane and butadiene[5]. Neither the experimental details, nor the yields of the intermediates were reported.

Our numerous experiments confirmed what could be read between the lines: the cycloaddition was far from being a clean reaction and the overall yield was very low. Moreover, the final amine **4a** was contaminated with the isomeric **4b**, indicating insufficient stereoselectivity of the Diels–Alder reaction.

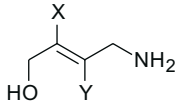


1a,1b

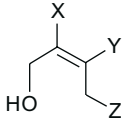


2a,2b

a, X = Me; Y = H
b, X = H; Y = Me



3a,3b



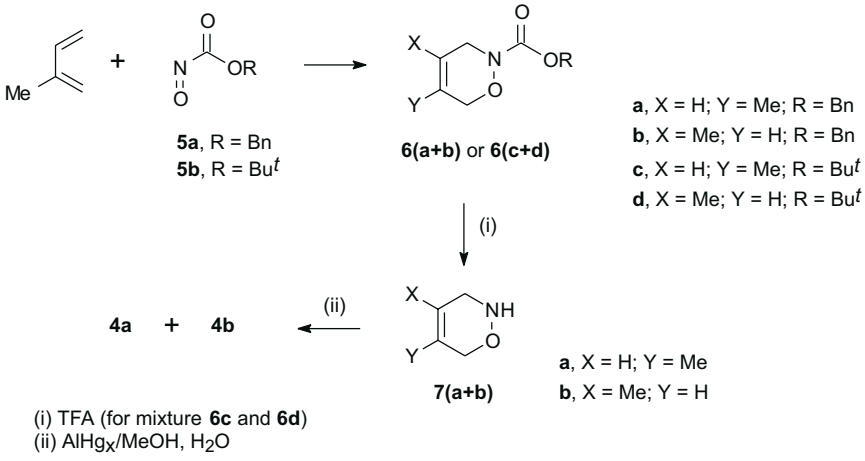
4a-4c

a, X = Me; Y = H; Z = NH_2
b, X = H; Y = Me; Z = NH_2
c, X = Me; Y = H; Z = NPhth

Shortly thereafter, Corse and Kuhnle[6] claimed that they isolated the (*Z*)-phthalimido derivative **4c** as a byproduct in their synthesis of **1a**. We have reproduced their procedure twice, except that chromatographic separation of the isomers was realized at the stage of the free **3a**. In the second separed substance the coupling J = 6.8 Hz between the olefinic methine and oxymethylene protons indicated a vicinal arrangement of these groups. Since both olefinic methyl and methine exhibited nuclear Overhauser effect (NOE) to both CH_2N and CH_2O protons, this compound was not **4a** but the other *E* isomer[7] **3b**. This was most probably also the case of a later modification[8], which used the same starting materials and intermediates. Only minute amounts of **4a** were isolated from the reaction of methyl (*Z*)-4-bromo-2-methylbut-2-enoate with sodium azide, followed by $LiAlH_4$ reduction of the 4-azido ester[9]. The Horner–Wadsworth–Emmons reaction in its Still–Gennari modification was succesfully applied in the highly stereoselective synthesis of **2a** by Evidente *et al.*[10]. Their well-elaborated five-step reaction pathway gives good results and is nowadays the only useful preparative method for **2a**. However, it is disqualified by drawbacks,

such as uncommon reagents, labor-consuming column chromatography after each step and scaling-up problems.

In the lasting need for a simpler access to **2a**, we turned back to the Diels–Alder strategy, but with an appropriate change of the nitroso component. The relatively new[11,12] metastable nitrosoformates **5** were the compounds of choice, possessing several advantages: easy *in situ* preparation from the corresponding *N*-hydroxycarbamates, clean reaction with isoprene and smooth conversion of the primary adducts **6** into the free 3,6-dihydro-2*H*-1,2-oxazines **7** (Scheme 1).



SCHEME 1

Our initial experiments were carried out with benzyl *N*-hydroxycarbamate[13], which was oxidized, in the presence of isoprene, with tetrabutylammonium periodate[14] to afford a mixture of **6a** and **6b** in a total yield of 48%. Structures of **6a** and **6b** were assigned to the products by determining which methylene (CH_2O or CH_2N) exhibited the **6a**/**6b** large vicinal coupling to the olefinic proton. NMR analysis showed, however, the isomer ratio 30 : 70, *i.e.* only a low proportion of the required **6a**. Hoping this ratio may be influenced by steric factors, we next used the more bulky dienophile **5b** and obtained a mixture of **6c** and **6d** in 52% yield and in a ratio 55 : 45. Removal of the ester grouping by trifluoroacetic acid then gave a mixture of dihydrooxazines **7a** and **7b** (as trifluoroacetates), which was further reduced without purification to the crude amino alcohols **4a** and **4b**. For this reduction, aluminium amalgam was found preferable to the previously used zinc in acetic acid[15]. The reduction mixture was

chromatographically separated into the individual **4a** and **4b** in the yields of 24.1 and 25.6% (based on the sum **6c** and **6d**), respectively. Both separated **4a** and **4b** were converted into the crystalline hemioxalates, which were finally condensed with 6-chloropurine to give the desired (*Z*)-zeatin (**2a**) and, in addition, the isomeric (*Z*)-isozeatin (**2b**). Only one reference to **2b** has been found in the literature. Its synthesis has been mentioned as being "in press" (ref.[8] in ref.[16]); however, the paper never appeared.

EXPERIMENTAL

Melting points are measured on Büchi-SMP 20 and are reported uncorrected. Chromatography was carried out on the Merck silica gel 60 (230–400 mesh) in chloroform–methanol–25% aqueous ammonia: 40 : 6 : 1 (A), 40 : 10 : 2 (B), 70 : 30 : 5 (C), 40 : 10 : 1 (D). HPLC analyses were performed on a reverse-phase column RP Select B (2 × 150 mm, 4 μm; Merck) using a Beckmann pump (model 128) and a Beckmann PDA detector (model 168). The solvent system was methanol–40 mM ammonium acetate buffer (pH 3.4); its linear gradient changed from the starting ratio 20 : 80 to 35 : 65 (15 min) and then to 50 : 50 (10 min). NMR spectra were measured on Varian VXR-400 or Inova-400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) at 25 or 30 °C in the indicated solvents (δ in ppm, *W* and *J* in Hz). Carbon signal multiplicity was determined by APT (Attached Proton Test). COSY and NOESY experiments used for the assignment were performed using the manufacturer's software.

Benzyl 5-Methyl-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylate (**6a**) and
Benzyl 4-Methyl-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylate (**6b**)

To a cool (–30 °C) solution of isoprene (1.02 g, 1.5 ml, 15 mmol) and benzyl *N*-hydroxycarbamate[13] (1.67 g, 10 mmol) in chloroform (70 ml), tetrabutylammonium periodate (4.33 g, 10 mmol) dissolved in chloroform (30 ml) was added dropwise within 45 min. The mixture was stirred for 1.5 h, reaching 0 °C and for another 1.5 h at room temperature. Then it was washed with 10% aqueous sodium thiosulfate (4 × 15 ml), water (30 ml), and dried over anhydrous sodium sulfate. Evaporation left a red oil (3.9 g), which was chromatographed on silica gel (100 g, chloroform) to give 1.12 g (48%) of a mixture of **6a** and **6b** as a colorless viscous oil. NMR showed the ratio **6a** : **6b** = 30 : 70. ^{1}H NMR ($CDCl_3$, 30 °C): δ major 1.739 (3 H, m, CH_3-C=); 4.021 (2 H, m, *W* = 8.9, CH_2N); 4.390 (2 H, m, *W* = 14.6, OCH_2); 5.223 (2 H, s, CH_2Ph); 5.536 (1 H, m, =CH); 7.297–7.406 (5 H, m, Ph); minor 1.663 (3 H, m, CH_3-C=); 4.110 (2 H, m, *W* = 12.9, CH_2N); 4.282 (2 H, m, *W* = 8.2, OCH_2); 5.218 (2 H, s, CH_2Ph); 5.524 (1 H, m, =CH); 7.297–7.406 (5 H, m, Ph). ^{13}C NMR ($CDCl_3$, 25 °C): δ major 19.66 q, 48.55 t, 67.68 t, 68.55 t, 118.02 d, 128.13 d, 128.23 d (2 C), 128.52 d (2 C), 130.13 s, 136.09 s, 155.51 s; minor 18.23 q, 44.76 t, 67.68 t, 71.65 t, 116.28 d, 128.13 d, 128.23 d (2 C), 128.52 d (2 C), 131.58 s, 136.09 s, 155.51 s.

tert-Butyl 5-Methyl-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylate (**6c**) and
tert-Butyl 4-Methyl-3,6-dihydro-2*H*-1,2-oxazine-2-carboxylate (**6d**)

Reverse addition: To tetrabutylammonium periodate (4.33 g, 10 mmol) in chloroform (60 ml) containing molecular sieve 4A (2.35 g), a solution of *tert*-butyl *N*-hydroxycarbamate[17] (1.33 g, 10 mmol) and isoprene (1.36 g, 20 mmol) in chloroform was added dropwise at –30 °C during 1 h. The temperature of the mixture was then slowly raised to 0 °C during 1.5 h and stirring was continued for another 1.5 h without cooling. The solution was concentrated to a small volume and chromatographed in the same way as in the previous paragraph. The fractions containing the product were combined, shaken with solid sodium thiosulfate (11 g) to remove pink color, filtered and evaporated. Yield 1.04 g (52%) of a yellowish oil; according to NMR, the ratio of **6c** : **6d** was 55 : 45. ^{1}H NMR ($CDCl_3$, 30 °C): δ major 1.501 (9 H, s, *t*-Bu); 1.628 (3 H, m, CH_3-C=); 4.030 (2 H, m, *W* = 14.1, CH_2N); 4.256 (2 H, m, *W* = 10.9, OCH_2); 5.516 (1 H, m, =CH); minor 1.507 (9 H, s, *t*-Bu); 1.736 (3 H, m, CH_3-C=); 3.941 (2 H, m, *W* = 10.9, CH_2N); 4.361 (2 H, m, *W* = 13.8, OCH_2), 5.531 (1 H, m, =CH). ^{13}C NMR ($CDCl_3$, 25 °C): δ major 18.75 q, 28.85 q (3 C), 44.81 t, 71.16 t, 81.48 s, 118.09 s, 155.63 s (C=O); minor 19.75 q, 28.83 q (3 C), 48.62 t, 68.00 t, 81.48 s, 116.59 d, 130.38 s, 155.63 s.

(*Z*-)-4-Amino-2-methylbut-2-en-1-ol (**4a**) and (*Z*)-4-Amino-3-methylbut-2-en-1-ol (**4b**)

A mixture of **6c** and **6d**, obtained as above (2.03 g, 10.2 mmol) was dissolved in dichloromethane (5 ml) and trifluoroacetic acid (6.5 ml) was added. The solution was set aside for 1 h at room temperature, evaporated *in vacuo* and repeatedly evaporated with methanol (2 × 30 ml). The resulting oily mixture of **7a** and **7b** (as trifluoroacetates) was dissolved in water (4.7 ml) and added to amalgamated aluminium foil (1.81 g, 67 mmol) in methanol (70 ml). The mixture was stirred vigorously for 3 h at ambient temperature, filtered, the inorganic matter was thoroughly washed with methanol and the combined filtrates evaporated to give a yellow oil (1.80 g). This was chromatographed on silica gel (120 g); solvent A, followed by B eluted **4b**, whereas for **4a** solvent C was used. The individual aminoalcohols were rechromatographed on a smaller silica gel column (50 g) in solvent B. The yields of pure compounds were: **4a**, 249 mg (24.1%), yellowish oil; **4b**, 264 mg (25.8%), yellow oil, crystallizes in the freezer. In addition to pure isomers, 75 mg (7.3%) of an unseparated mixture **4a** and **4b** was obtained.

4a: ^{1}H NMR ($CDCl_3$, 25 °C): δ 1.732 (3 H, dt, *J* = 1.5, 0.8, CH_3-C=); 3.280 (2 H, dq, *J* = 7.1, 0.8, CH_2N); 3.576 (3 H, br s, OH + NH_2); 4.001 (2 H, br s, OCH_2); 5.357 (1 H, tqt, *J* = 7.2, 1.5, 0.8, =CH). ^{13}C NMR ($CDCl_3$, 25 °C): δ 21.75 q, 38.06 t, 61.04 t, 125.53 d, 139.22 s.

4b: ^{1}H NMR ($CDCl_3$, 25 °C): δ 1.766 (3 H, dt, *J* = 1.5, 0.9, CH_3-C=); 3.275 (2 H, br s, CH_2N); 4.036 (2 H, dq, *J* = 7.0, 0.9, OCH_2); 5.544 (1 H, tqt, *J* = 7.0, 1.5, 0.9, =CH). ^{13}C NMR ($CDCl_3$, 25 °C): δ 22.24 q, 41.53 t, 57.79 t, 126.98 d, 138.06 s.

The free bases **4a** and **4b** were converted into hemioxalates according to ref.[10].

4a·0.5 $(CO_2H)_2$: yield 306 mg (85%), m.p. 182–184 °C (dec.), (ref.[10], m.p. 183.5–185 °C). ^{1}H NMR (D_2O, 30 °C): δ 1.848 (3 H, dt, *J* = 1.1, 0.75, CH_3-C=); 3.686 (2 H, dq, *J* = 7.5, 1.1, CH_2N); 4.163 (2 H, d, *J* = 0.75, OCH_2); 5.466 (1 H, ttq, *J* = 7.5, 1.1, 0.75). ^{13}C NMR (D_2O, 30 °C): δ 23.48 q, 39.28 t, 62.76 t, 121.01 d, 145.64 s, 176.28 s (C=O).

4b·0.5 $(CO_2H)_2$: yield 315 mg (82.5%), m.p. 162–165 °C (dec.); from water–ethanol (1 : 10), m.p. 168–170 °C (dec.). For $C_6H_{12}NO_3$ (146.2) calculated: 49.30% C, 8.28% H, 9.58% N; found: 49.25% C, 8.41% H, 9.50% N. ^{1}H NMR (D_2O, 30 °C): δ 1.865 (3 H, dt, *J* =

1.6, 0.8, CH_3-C=); 3.685 (2 H, d, *J* = 0.8, CH_2N); 4.164 (2 H, dq, *J* = 6.0, 1,1, OCH_2); 5.785 (1 H, ttq, *J* = 6.0, 1.1, 1.6, =CH). ^{13}C NMR (D_2O, 30 °C): δ 23.43 q, 42.07 t, 59.96 t, 133.14 d, 133.78 s, 176.23 s (C=O).

(*Z*)-Zeatin (**2a**)

The suspension of **4a**·0.5 $(CO_2H)_2$ (146 mg, 1 mmol) and 6-chloropurine (232 mg, 1.5 mmol) in propan-1-ol (5 ml) containing triethylamine (405 mg, 4 mmol) was stirred under argon for 5 h at 90 °C. The mixture was evaporated *in vacuo* and chromatographed on a silica gel column (3 × 14 cm) in system D, giving **2a** (189 mg; 80.6%, based on the starting oxalate), m.p. 212–214 °C; after recrystallization from ethanol, m.p. 214–215 °C (m.p. in ref.[4], 206–208 °C; in ref.[10], 212–214 °C). The HPLC purity was over 99%. ^{1}H NMR (DMSO-d_6, 30 °C): δ 1.703 (3 H, bs, =C-CH_3); 4.036 (2 H, *J* = 5.2, CH_2O); 4.131 (2 H, m, NCH_2); 4.755 (1 H, t, *J* = 5.2, OH); 5.347 (1 H, t, *J* = 6.8, =C-H); 7.565 (1 H, bs, NH); 8.064 and 8.160 (1 H each, s, purine protons).

(*Z*)-Isozeatin (**2b**)

A suspension of **4b**·0.5 $(CO_2H)_2$ (44 mg, 0.3 mmol) and 6-chloropurine (30.8 mg, 0.2 mmol) in propan-1-ol (2 ml) containing triethylamine (141 mg, 1.4 mmol) was stirred for 12 h at 90 °C, evaporated, dissolved in 50% methanol and applied on a small column of Dowex 50 × 8 in $[H]^+$ cycle. The product, together with unreacted **4b**, was eluted with 1 : 1 mixture of methanol–3% aqueous ammonia and recrystallized from ethanol (1 ml). Yield, 29 mg (66%, calculated on 6-chloropurine, 44% on **4b**), m.p. 216–217 °C. HPLC purity was better than 99%. For $C_{10}H_{13}N_5O$ (219.2) calculated: 54.78% C, 5.98% H, 31.95% N; found: 54.67% C, 6.08% H, 32.11% N. ^{1}H NMR (DMSO-d_6, 30 °C): δ 1.684 (3 H, dt, *J* = 1.2, 1.2, =C-CH_3); 3.417 (2 H, bs, NCH_2); 4.085 (2 H, d, *J* = 6.6, OCH_2); 4.147 (1 H, bs, NH); 5.424 (1 H, ttq, *J* = 6.6, 1.0, 1.2, =C-H); 8.088 and 8.161 (1 H each, s, purine protons).

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7. Data for **3b**·0.5 $(CO_2H)_2$: m.p. 169–171 °C. ^{1}H NMR (D_2O, 30 °C): δ 1.772 (3 H, m, CH_3-C=); 3.583 (2 H, br s, CH_2N); 4.204 (2 H, dqt, *J* = 6.8, 1.3, 0.8, OCH_2); 5.675 (1 H,

tqt, *J* = 6.8, 1.4, 1.3, =CH). ^{13}C NMR (D_2O, 30 °C): δ 16.69 q, 48.67 t, 60.33 t, 130.68 d, 134.18 s, 176.30 s (C=O).

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