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New approach to asymmetrically substituted methoxypyrazines, derivatives of wine flavors

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ABSTRACT

An original synthetic route to asymmetrically substituted methoxypyrazine (MP) derivatives is described. The first step of the synthesis is achieved by condensation of 1,2-aminoalcohols with Boc-protected aliphatic aminoacids followed by cycloimine formation and aromatization via chlorination. Introduction of methoxy group was then achieved by alkoxy-de-halogenation. The use of primary or secondary aminopropanol enabled the direct and selective introduction of methyl group, in 5- or 6-position, which can be easily functionalized. Aromatization of diketopiperazine, prepared from L-valine and L-glutamic acid dimethyl ester, made possible a direct introduction of a functionalized alkyl chain. These reactions are also suitable for the synthesis of naturally-occurring MPs such as wine flavor components and biologically active substances.

1. Introduction

Methoxypyrazines (MP), namely 2-isobutyl-3-methoxypyrazine (IBMP), 2-isopropyl-3-methoxy pyrazine (IPMP), and 2-*sec*-isobutyl-3-methoxypyrazine (SBMP) (Fig. 1), have been identified in a wide range of materials of vegetal origin[1,2] and are particularly known to be responsible for the very characteristic green, herbaceous, or vegetative aromas of Sauvignon blanc and Cabernet Sauvignon wines.[3–5] The presence of these compounds may be very pleasing in some dry white wines, favorably contributing to their herbal/grassy character, but is often undesirable in red wines. MP levels can have a significant impact on wine quality and, as a result, on its market value.

MPs are extremely potent flavor compounds, with particularly low detection threshold values (1–2 ng/L in water and 5–15 ng/L in wine and model solution) and the rejection threshold seems to be around 30 ng/L in water.[3,6,7] The final MP level in wine depends on grape variety and ripening, climate parameters and wine making practices.[5,8,9] During the development of new analytical methods for the control of MP level, we have investigated different synthetic approaches to a variety of MP analogues in order to synthesize MPs functionalized with a carboxylic function at 5- or 6-position for coupling to proteins, and for developing specific antibodies.

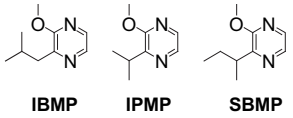

Figure 1. 2-Alkyl-3-methoxypyrazines present in wine.

In the literature, several alternative syntheses of MP derivatives have been proposed (Scheme 1). In most of the approaches, an α-aminoacid amide is condensed with 1,2-dicarbonyl compounds and good yields were reported when a symmetrical bicarbonyl reagent was used.[6,10–15] This pathway is not very appropriate for the preparation of mono-substituted MP derivatives at positions 5- or 6- because of the formation of different isomers, which are difficult to separate.[14,15] Another approach including the aromatization of a cyclic dipeptide by chlorination and the subsequent introduction of methoxy group by alkoxy-de-halogenation, gave a low overall yield because of the non-selectivity of chlorination.[16,17] Different MP analogues for flavor use were also synthesized by the condensation of methyl pyruvate and ethylenediamine.[18] MPs can be also obtained by the direct- or side chain alkylation and subsequent nuclear chlorination of methylpyrazine.[19,20] Finally, several novel naturally-occurring MPs were synthesized by subsequent methoxylation and Grignard reagent alkylation of 2,6-dichloropyrazines obtained from pyrazine-*N*,*N*′-dioxide.[21] In the same work, symmetrically substituted 2,5-dialkyl MPs were prepared by dimerisation of α-azido ketones. This method cannot be applied to the preparation of asymmetrically substituted MPs because the dimerisation procedure yielded a mixture of regio-isomers inseparable by column chromatography.[21] Recently, alternative one-pot pyrazine preparation involving manganese dioxide-based tandem oxidation of

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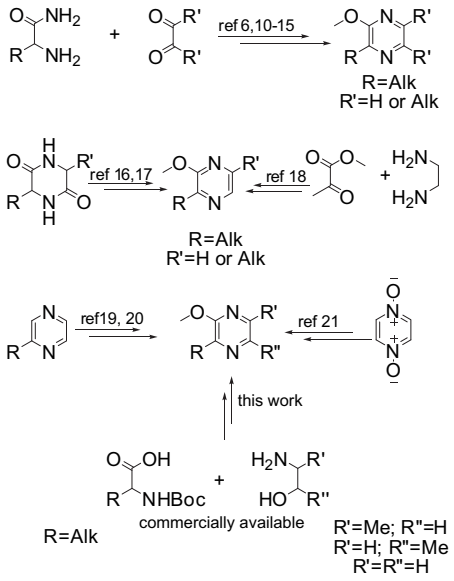


Scheme 1. Synthetic approaches to MP derivatives.

α-hydroxyketones, in situ trapping with 1,2-diamines, and basic aromatization gave relatively good yields[22] and could be applied to MP synthesis. In most cases, the multi-step preparation of starting materials is needed, leading to considerably decreased overall yields. Moreover, these methods are not very useful for the synthesis of asymmetrically substituted functional MP derivatives.

In this study, we describe a new synthetic pathway to asymmetrical MP derivatives based on the condensation of 1,2-aminoalcohols with Boc-protected aliphatic aminoacids allowing the direct introduction of alkyl (*i*-Bu, *i*-Pr, *s*-Bu) groups as well as the unique site for the subsequent introduction of methoxy group via a chlorination step (Scheme 1). The direct and selective introduction of a methyl group in 5- or 6-position enables an easy access to an IBMP hapten for further development of new immunological methods for MP analysis.

2. Results and discussion

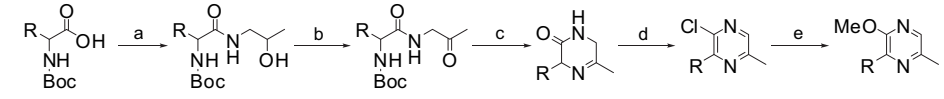
2.1. Synthesis of asymmetrically substituted MP derivatives

The synthesis began with the amide bond formation between 1-aminopropan-2-ol and Boc-protected leucine **1** or valine **2** for the preparation of IBMP and IPMP parent compounds, respectively (Scheme 2). The best yields were obtained with *N*-hydroxysuccinimide compared to HOBt (1-hydroxybenzotriazole) activation. The oxidation of secondary alcohols **3** and **4** afforded the corresponding ketones **5** and **6** in 75–80% yields. For this step TEMPO (tetramethylpiperidinyloxyl radical) mediated oxidation with household bleach was chosen on green and low cost criteria.[23,24] The reaction was generally complete within 45 min and the crude product was obtained with a sufficient purity to be used directly for Boc-deprotection, which was achieved by HCl treatment. The quantitative cycloimine formation occurred in anhydrous conditions to afford **7** and **8** in 88–90% overall yield from the corresponding ketones. The aromatization of dihydropyrazinones **7** and **8** via chlorination was achieved by heating with phosphorus oxychloride and phosphorus pentachloride. Nevertheless, even if in our case the chlorination was regioselective, this reaction remains the limiting step because of the need for purification (by vacuum distillation or by flash chromatography) leading to moderate yields (40–45%). All attempts to improve the yield were unsuccessful. Finally, the monochloro-derivatives **9** and **10** were treated with methanolic sodium methoxide to afford the target 6-methyl substituted MP derivatives **11** and **12** in 87–90% yield.

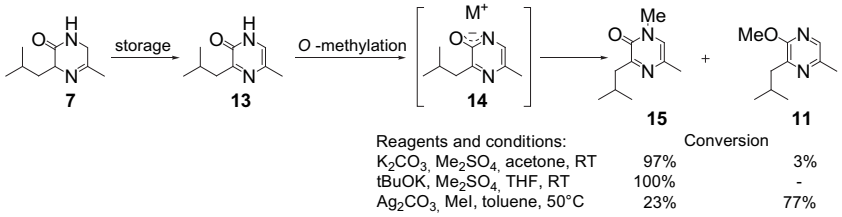
It was observed that the intermediate **7** was dehydrogenated to the corresponding pyrazinone **13** during storage over several days in the presence of atmospheric oxygen (Scheme 3). As **13** enables the formation of an ambident anion **14**, the methylation reaction can give both O- and N-alkylation.[25] Only N-methylation was observed when potassium salts and dimethyl sulfate were used. The O-methylation was favored when anion **14** was generated by treatment with silver carbonate. The site of methylation was assigned on the basis of ^{1}H and ^{13}C NMR spectra and HMBC correlations (Fig. 2). The signals at 3.47 and at 3.90 ppm were attributed to *N*-methyl and *O*-methyl groups, respectively. IR spectrum of **15** showed strong C=O absorption at 1644 cm^{-1}, which is not present in the IR spectrum of **11**. It seemed to be important for us to clarify this point because of already-described wrong structure assignment.[12] In our hands, the *O*-methylation was never selective and the mixture of **11** and **15** was difficult to separate; only enriched fractions were obtained either by chromatography or vacuum distillation.

To obtain 5-methyl MP derivatives, reactions of Boc-protected aliphatic aminoacids with 2-aminopropan-1-ol were also carried out (Scheme 4). In this case primary alcohols **17**–**19** obtained in high yield were oxidized to the corresponding aldehydes **20**–**22**. These aldehydes turned out to be very unstable and were easily cyclised in dichloromethane over 24 h to give the corresponding Boc-protected 3-oxo-3,4-dihydropyrazines **23**–**25**.

For further functionalisation, compound **23** was converted (Scheme 5) to the corresponding asymmetrically substituted methoxy-derivative **27** (see below Scheme 7). In the first step, Boc-



Scheme 2. Reagents and conditions: (a) 1-aminopropan-2-ol, DCC, NHS (or HOBt), CH_2Cl_2; (b) household bleach, TEMPO, $NaHCO_3$, CH_2Cl_2; (c) i. HCl, CH_2Cl_2, ii. Et_3N, Et_2O, 4 Å molecular sieves; (d) $POCl_3$, PCl_5; (e) NaOMe, MeOH.



Scheme 3. Methylation of pyrazinone.

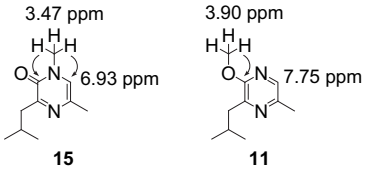
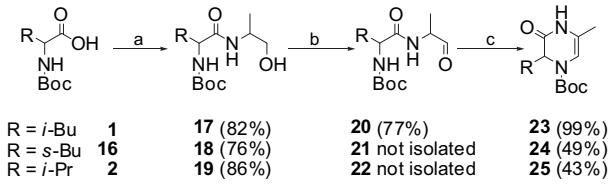
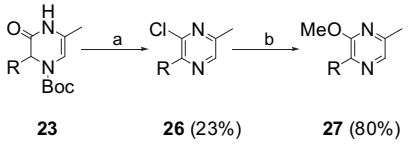


Figure 2. ^{1}H NMR and HMBC correlations for *N*- and *O*-methylated pyrazine derivatives.



Scheme 4. Reagents and conditions: (a) 2-aminopropan-1-ol, DCC, NHS, CH_2Cl_2; (b) household bleach, TEMPO, $NaHCO_3$, CH_2Cl_2; (c) spontaneous cyclisation, 24–36 h.

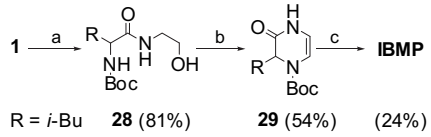


Scheme 5. Reagents and conditions: (a) $POCl_3$, PCl_5; (b) NaOMe, MeOH.

deprotection, chlorination and aromatization reactions were achieved simultaneously with classical chlorinating agents $POCl_3$–PCl_5 and the chloro-derivatives **26** was obtained in 23% yield after purification.

In both synthetic pathways (Schemes 2 and 4) the yields and purities of the intermediates were generally better for *i*-Bu and *s*-Bu series compared to the *i*-Pr derivatives.

In a similar manner, our new synthetic approach was applied to the synthesis of naturally-occurring IBMP. The coupling Boc–leucine with aminoethanol gave the alcohol **28**, which was transformed to **29** without any isolation of intermediates. In the same manner as above, **29** was chlorinated and methoxylated. After only one purification procedure by distillation, IBMP was obtained in 10% overall yield (Scheme 6), that is, comparable to previously described IBMP preparation.[11]



Scheme 6. Reagents and conditions: (a) 2-aminoethanol, DCC, NHS, CH_2Cl_2; (b) i. household bleach, TEMPO, $NaHCO_3$, then spontaneous cyclisation during the storage in CH_2Cl_2 for 24 h; (c) i. $POCl_3$, PCl_5; ii. NaOMe, MeOH.

2.2. Introduction of a spacer bearing a carboxylic function

For development of immunological assays of MPs in wine and grape, the synthesis of functionalized derivatives bearing a carboxylic function is required for covalent coupling to carrier proteins. The 5-position was first considered as the best choice for anchorage because it is the most remote site from the discriminating 2-alkyl substituents. The synthesis of IBMP hapten **32** is described in Scheme 7.

Alkylation of the methyl group of **27** at the benzylic position was already studied by Otah;[17] the benzylic carbanion was generated using potassium diisopropylamide (KDA) and then quenched with 2-bromoethyl tetrahydropyranyl ether. However, in our hands, using KDA with bromoacetaldehyde diethylacetal was unsuccessful. Therefore, we next examined the benzylic alkylation with superbases. Heavy alkali metal alkoxides have been described as dramatically increasing the reactivity of organolithium compounds (more than 10^6).[26,27] The alkylation of **27** was achieved in the presence of *i*-Pr_2NH/*n*-BuLi/*t*-BuOK and the expected alkylated pyrazine **30** was obtained in 70% yield. The terminal carboxylic acid function was generated by the hydrolysis of acetal **30**, under acidic conditions (trifluoroacetic acid/chloroform/water),[28] followed by an oxidation with potassium permanganate, under basic conditions to provide the target hapten **32** in 42% yield from **27**.

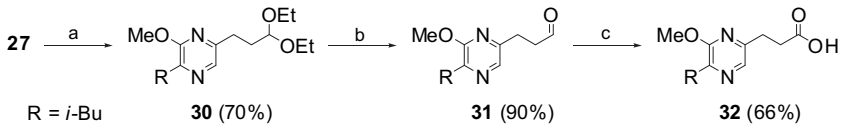
Given that the synthesis of *i*-Pr chloro-derivative required for IPMP hapten preparation gave a modest yield, we attempted a direct introduction of a linker functionalized with a carboxylic group via the aromatization of a cyclic dipeptide *cyclo*(Val-Glu) **34** (Scheme 8).

The diketopiperazine **34** was prepared from L-valine and L-glutamic acid dimethyl ester hydrochloride[29] using a reported procedure.[30] The chlorination of **34** could not be achieved regioselectively and resulted in a mixture of a dichloropyrazine **36** and both monochloropyrazines **36** and **37**. The isolation of 3-chloroderivative **37** was performed by preparative HPLC and gave a yield of 7%. Finally the methoxylation and deprotection of terminal carboxylic function were achieved by treatment with sodium methoxide and afforded the IPMP hapten **38** in 81% yield.

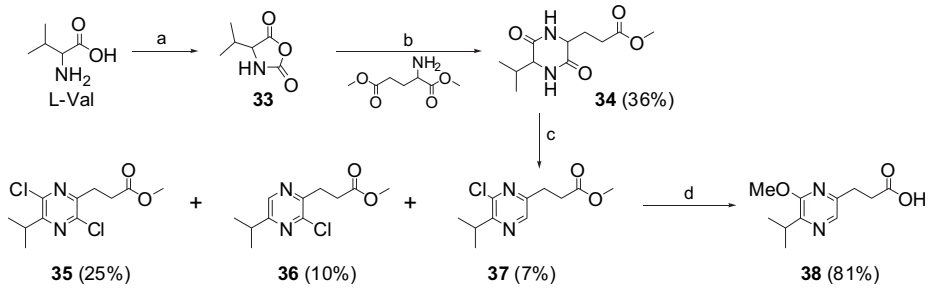
Both haptens **32** and **38** were coupled to bovine serum protein (BSA) to afford the corresponding immunogenic hapten–protein conjugates for antibody production.

3. Conclusion

In this study, we have described an original synthetic route to asymmetrically substituted MP derivatives based on the condensation of 1,2-aminoalcohols with Boc-protected aliphatic amino-acids. The pathway utilized commercially available inexpensive starting materials and involved three to five steps with a few purifications. The direct and selective introduction of methyl group in 5 or 6 position on the pyrazine ring was performed using primary or secondary aminopropanols, respectively. The methyl group was then easily functionalized to afford an IBMP hapten for further use in the development of immunological methods for MP quantification in agricultural commodities. Our synthetic pathway was also validated by the preparation of naturally-occurring IBMP and proved to be competitive compared to previously published syntheses.[11]



Scheme 7. Reagents and conditions: (a) KDA, BuLi, THF, $BrCH_2CH(OEt)_2$; (b) CF_3COOH, $CHCl_3$, H_2O; (c) $KMnO_4$, Na_2CO_3, H_2O.



Scheme 8. Reagents and conditions: (a) $ClCOOCCl_3$, THF; (b) Et_3N, THF, CH_2Cl_2; (c) i. $POCl_3$, PCl_5, ii. HPLC separation; (d) NaOMe in MeOH, then H_2O.

Furthermore, our approach presents a special interest for the synthesis of other MP derivatives like recently identified novel MP metabolites,[31] as well as for the synthesis of known biologically active compounds like Arglecin[17] or Argvalin.[16] It could be useful for the preparation of deuterated standards[12,32] and for the synthesis of pyrazine-like flavoring agents.

4. Experimental

4.1. General

Chemical reagents and solvents were purchased from Sigma Aldrich Chemical Co. (Saint Quentin Fallavier, France), Alfa Aesar. (Bisheim, France), or Acros Organics France (Noisy, France). All moisture sensitive reactions were performed under an argon atmosphere in ovendried or flame-dried glassware.

Instruments. Melting points were determined with a Stuart Scientific melting point apparatus SMP3 and are uncorrected. ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-300 FT (^1H, 300 MHz; ^{13}C, 75 MHz) or with a Bruker AC-250 (^1H, 250 MHz; ^{13}C, 63 MHz) using TMS as an internal standard. The chemical shifts (δ) and coupling constants (J) are respectively expressed in parts per million and hertz. IR spectra were recorded with a Perkin–Elmer Paragon 1000 FTIR spectrophotometer. Merck silica gel 60 (70–230 mesh, 0.063–0.200 mm) was used for flash chromatography. Thin-layer chromatography (TLC) was performed using TLC plates (0.25 mm, particle size 15 μm, pore size 60 Å) purchased from SDS (Peypin, France). TLC plates were run in the same solvent system as flash chromatography. The spots were visualized with a UV lamp. Mass spectra were acquired by the CESAMO (Bordeaux, France) on a QStar Elite mass spectrometer (Applied Biosystems). The instrument was equipped with an electrospray ionization (ESI) source and spectra were recorded in the positive mode. The electrospray needle was maintained at 4500 V and operated at room temperature. Samples were introduced by injection through a 10 μL sample loop into a 200 μL min^{-1} flow of methanol from the LC pump.

4.2. Chemical synthesis

*4.2.1. N^2-(tert-Butoxycarbonyl)-N-(2-hydroxypropyl)leucinamide (**3**).* A solution of DCC (2.88 g, 14 mmol) in dichloromethane (50 mL) was added dropwise to an ice-cooled solution of *N*-(*tert*-butoxycarbonyl)leucine (3 g, 13 mmol) and *N*-hydroxysuccinimide (1.61 g, 14 mmol) in dichloromethane (50 mL). The reaction mixture was allowed to warm at room temperature, stirred for 4 h and filtered. A solution of 1-aminopropan-2-ol (2.9 g, 39 mmol) in dichloromethane (10 mL) was added to the filtrate. The mixture was stirred overnight and washed with water (100 mL). The aqueous layer was extracted with dichloromethane (2×50 mL). Combined organic layers were washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give **3** as a brown oil (3.37 g; 90%). It was used for the next step without any purification. ^1H NMR ($CDCl_3$, δ (ppm)): 0.90 (d, ^3J=7 Hz, 3H, $CH(CH_3)_2$), 0.92 (d, ^3J=7 Hz, 3H, $CH(CH_3)_2$), 1.15 (d, ^3J=7 Hz, 3H, $CHOHCH_3$), 1.40 (s, 9H, $C(CH_3)_3$), 1.60 (m, 1H, $CH(CH_3)_2$), 1.64 (m, 2H, $CH_2CH(CH_3)_2$), 3.15 (m, 1H, $NHCH_2$),3.41 (m, 1H, $NHCH_2$), 3.90, (1H, CHOH), 4.10 (m, 1H, NCHCO), 4.99 (s, 1H, OH), 5.25 (s, 1H, NH), 7.05 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.8 (CH_3), 21.1 (CH_3), 22.2 (CH_3), 25.2 (CH), 28.6 (CH_3), 41.6 (CH_2), 47.3 (CH_2), 52.6 (CH), 64.5 (CH), 79.9 (C), 156.3 (C), 174.4 (C). IR (ν (cm^{-1})): 3437, 1539. HRMS (ESI^+) m/z calcd for $C_{14}H_{28}N_2O_4$, 311.1942 $[M+Na]^+$; found, 311.1948.

The procedure described above for **3** was employed for the synthesis of isopropyl analogue **4** using Boc-valine and 1-aminopropan-2-ol; compounds **17**–**19** and **29** were synthesized likewise using corresponding Boc-protected aminoacid and 2-aminopropan-1-ol or 2-aminoethanol, respectively.

*4.2.2. N^2-(tert-Butoxycarbonyl)-N-(2-hydroxypropyl)valinamide (**4**).* Brown oil, yield 75%. ^1H NMR ($CDCl_3$, δ (ppm)): 0.90 (d, ^3J=7 Hz, 3H, $CH(CH_3)_2$), 0.93 (d, ^3J=7 Hz, 3H, $CH(CH_3)_2$), 1.18 (d, ^3J=7 Hz, 3H, $CHOHCH_3$), 1.43 (s, 9H, $C(CH_3)_3$), 2.10 (m, 1H, $CH(CH_3)_2$), 3.11 (m, 1H, CH_2), 3.37 (m, 1H, CH_2), 3.93–3.88 (m, 2H, NHCHCO, CHOH), 5.16 (s, 1H, NH), 6.66 (m, 2H, NH, OH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 18.3 (CH_3), 19.7 (CH_3), 21.0 (CH_3), 28.7 (CH_3), 31.1 (CH), 47.4 (CH_2), 60.8 (CH), 67.3 (CH), 80.6 (C), 156.6 (C), 173.2 (C). IR (ν (cm^{-1})): 3430, 1539. HRMS (ESI^+) m/z calcd for $C_{13}H_{26}N_2O_4$, 297.1784 $[M+Na]^+$; found, 297.1791.

*4.2.3. N^2-(tert-Butoxycarbonyl)-N-(2-hydroxy-1-methylethyl)leucinamide (**17**).* Brown oil, yield 82%. ^1H NMR ($CDCl_3$, δ (ppm)): 0.87 (d, ^3J=7 Hz, 3H, $CH(CH_3)_2$), 0.89 (d, ^3J=7 Hz, 3H, $CH(CH_3)_2$), 1.18 (d, ^3J=7 Hz, 3H, $CHCH_3$), 1.40 (s, 9H, $C(CH_3)_3$), 1.65 (m, 3H, CH_2CH, CH_2CH), 3.40 (m, 1H, CH_2OH), 3.58 (m, 2H, CH_2OHCH, CH_2OH), 4.00 (m, 2H, OH, COCHNH), 5.35 (m, 1H, NH), 6.80 (m, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 16.8 (CH_3), 22.2 (CH_3), 23.5 (CH_3), 25.0 (C), 28.8 (CH_3), 41.5 (CH_2), 47.5 (CH), 53.6 (CH), 66.9 (CH_2), 80 (C), 156.2 (C), 173.0 (C). IR (ν (cm^{-1})): 3435, 1648. HRMS (ESI^+) m/z calcd for $C_{14}H_{28}N_2O_4Na$, 311.1941 $[M+Na]^+$; found, 311.1947.

*4.2.4. N^2-(tert-Butoxycarbonyl)-N-(2-hydroxy-1-methylethyl)isoleucinamide (**18**).* Brown oil, yield 76%. ^1H NMR ($CDCl_3$, δ (ppm)): 0.85 (t, ^3J=7 Hz, 3H, CH_2CH_3), 0.89 (d, ^3J=7 Hz, 3H, $CHCH_3$), 1.11 (d, ^3J=7 Hz, 3H, $NHCHCH_3$), 1.37 (s, 9H, $C(CH_3)_3$), 1.65 (m, 3H, CH_2CH, CH_2CH), 3.50 (m, 2H, CH_2OH), 3.86 (m, 2H, CH_2OHCH, NHCHCO), 5.47 (m, 1H, NH), 5.50 (m, 1H, NH), 7.01 (m, 1H, OH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 11.1 (CH_3), 15.3 (CH_3), 16.8 (CH_3), 24.7 (CH_2), 28.2 (CH_3), 37.0 (CH), 47.6 (CH), 60.0 (CH), 66.2 (CH_2), 79.7 (C), 155.9 (C), 172.2 (C). IR (ν (cm^{-1})): 3450, 1539. HRMS (ESI^+) m/z calcd for $C_{14}H_{28}N_2O_4Na$, 311.1941 $[M+Na]^+$; found, 311.1949.

*4.2.5. N^2-(tert-Butoxycarbonyl)-N-(2-hydroxy-1-methylethyl)valinamide (**19**).* Brown oil, yield 86%. ^1H NMR ($CDCl_3$, δ (ppm)): 0.90 (d, ^3J=7 Hz, 3H, $CHCH_3$), 0.94 (d, ^3J=7 Hz, 3H, $CHCH_3$), 1.16 (d, ^3J=7 Hz, 3H, CH_3), 1.43 (s, 9H, $C(CH_3)_3$), 2.10 (m, 1H, CH), 3.50 (m, 2H, CH_2OH), 3.85 (m, 1H, CH), 4.10 (m, 1H, CH), 5.30

(m, 2H, NH), 6.50 (m, 1H, OH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 14.2 (CH_3), 16.8 (CH_3), 17.4 (CH_3), 28.3 (CH_3), 33.7 (CH), 47.8 (CH), 66.3 (CH), 66.7 (CH), 80.1 (C), 156.1 (C), 172.1 (C). IR (ν (cm^{-1})): 3436, 1647. HRMS (ESI^+) *m*/*z* calcd for $C_{13}H_{27}N_2O_4$, 275.1965 $[M+H]^+$; found, 275.1978.

*4.2.6. N^2-(tert-Butoxycarbonyl)-N-(2-hydroxyethyl)leucinamide (**28**).* 2-Aminoethan-1-ol was used. Brown oil, yield 81%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.86 (d, ^3J=7 Hz, 3H, CHCH_3), 0.89 (d, ^3J=7 Hz, 3H, CHCH_3), 1.37 (s, 9H, CH_3), 1.50 (m, 3H, CH_2CH), 3.40 (m, 2H, CH_2), 3.64 (m, 2H, CH_2), 4.10 (m, 1H, CH), 4.73 (s, 1H, OH), 5.45 (m, 1H, NH), 7.26 (m, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 22.3 (CH_3), 23.3 (CH_3), 25.1 (CH), 28.7 (CH_3), 41.9 (CH_2), 42.7 (CH_2), 53.7 (CH), 61.9 (CH_2), 80.4 (C), 156.4 (C), 174.4 (C). IR (ν (cm^{-1})): 3530, 1738.

*4.2.7. N^2-(tert-Butoxycarbonyl)-N-(2-oxopropyl)leucinamide (**5**).* $NaHCO_3$ (6 g, 71 mmol) and TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, free radical, 0.2 g, 1.3 mmol) were added to an ice-cooled solution of **3** (7.6 g, 26 mmol) in dichloromethane (100 mL). Household bleach (4.8% NaOCl, 100 mL) was then added dropwise and the resulting mixture was stirred at room temperature overnight. The product was extracted with dichloromethane (3×100 mL). Combined organic layers were washed with water (3×50 mL) and brine (50 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give **5** as an oil (7 g, 90%). It was used for the next step without any purification. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.93 (2d, ^3J=7 Hz, 6H, CH(CH_3)$_2$), 1.38 (s, 9H, C(CH_3)$_3$), 1.58–1.40 (m, 3H, CH_2CH(CH_3)$_2$), 2.14 (s, 3H, COCH_3), 4.08 (d, ^3J=5 Hz, 2H, COCH_2), 4.18 (m, 1H, NHCHCO), 5.27 (s, 1H, NH), 7.18 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 21.8 (CH_3), 22.9 (CH_3), 24.6 (CH), 27.1 (CH_3), 28.2 (CH_3), 41.4 (CH_2), 49.7 (CH_2), 52.9 (CH), 79.8 (C), 155.7 (C), 173.2 (C), 203.2 (C). IR (ν (cm^{-1})): 1539. HRMS (ESI^+) *m*/*z* calcd for $C_{14}H_{26}N_2O_4Na$, 309.1790 $[M+Na]^+$; found, 309.1783.

The NaOCl/TEMPO system was used for the oxidation of primary and secondary alcohols **4**, **17**–**19**, and **28** into corresponding aldehydes and ketones **6**, **20**–**22**, and **29**.

*4.2.8. N^2-(tert-Butoxycarbonyl)-N-(2-oxopropyl)valinamide (**6**).* Colorless oil, yield 80%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.87 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 0.93 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 1.41 (s, 9H, C(CH_3)$_3$), 2.11 (m, 1H, CH(CH$_3$)$_2$), 2.17 (s, 3H, CH_3CO), 4.01 (m, 1H, COCHNH), 4.13 (d, ^3J=5 Hz, 2H, COCH_2), 5.19 (s, 1H, NH), 6.87 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 17.6 (CH_3), 19.2 (CH_3), 27.3 (CH_3), 28.3 (CH_3), 30.9 (CH_2), 49.6 (CH_2), 59.7 (CH), 79.9 (C), 155.8 (C), 171.9 (C), 202.8 (C). IR (ν (cm^{-1})): 2929, 1539. HRMS (ESI^+) *m*/*z* calcd for $C_{13}H_{24}N_2O_4Na$, 295.1628 $[M+Na]^+$; found, 295.1637.

*4.2.9. N^2-(tert-Butoxycarbonyl)-N-(1-methyl-2-oxoethyl)-leucinamide (**20**).* TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, free radical, 0.02 g, 0,135 mmol) and saturated $NaHCO_3$ solution (27,5 mL) were added to an ice-cooled solution of **17** (3.88 g, 13.45 mmol) in dichloromethane (50 mL). Household bleach (4.8% NaOCl, 35 mL) was then added dropwise and the resulting mixture was stirred at room temperature during 4 h. Organic layer was washed with saturated NH_4Cl solution (3×50 mL), dried over magnesium sulfate, and filtered. Organic layer was concentrated under reduced pressure to give **20** as an oil (2.77 g, 77%). ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.89 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 0.90 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 1.35 (d, ^3J=7 Hz, 3H, CHCH_3), 1.45 (s, 9H, C(CH_3)$_3$), 1.60 (m, 3H, CH_2, CH$_2$CH), 4.17 (m, 1H, NHCHCO), 4.42 (m, 1H, NHCHCHO), 5.11 (m, 1H, NH), 7.00 (m, 1H, NH), 9.50 (s, 1H, CHO). HRMS (ESI^+) *m*/*z* calcd for $C_{14}H_{26}N_2O_4Na$, 309.1784 $[M+Na]^+$; found, 309.1796.

The aldehydes **20**–**22** cyclised spontaneously during storage in dichloromethane over 24 h to give the corresponding Boc-protected 3,4-dihydropyrazines **23**–**25** in quantitative yield.

*4.2.10. tert-Butyl 2-isobutyl-5-methyl-3-oxo-3,4-dihydropyrazine-1(2H)-carboxylate (**23**).* Brown oil, yield 99%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.95 (t, ^3J=7 Hz, 6H, CH(CH_3)$_2$), 1.35 (m, 3H, CH(CH$_3$)$_2$, CH_2), 1.48 (s, 9H, C(CH_3)$_3$), 1.85 (s, 3H, CH_3), 4.80–4.58 (m, 1H, CHN), 6.00 (s, 1H, C=CH), 8.10 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 17.0 (CH_3), 21.0 (CH_3), 22.0 (CH), 28.1 (CH_3), 41.1 (CH_2), 54.9 (CH), 80.9 (C), 103.7 (C), 118.5 (CH), 152.3 (C), 168.8 (C).

*4.2.11. tert-Butyl 2-sec-butyl-5-methyl-3-oxo-3,4-dihydropyrazine-1(2H)-carboxylate (**24**).* Brown oil, yield 49% from **18**. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.84 (t, ^3J=7 Hz, 3H, CH$_2$CH_3), 0.91 (d, ^3J=7 Hz, 3H, CHCH_3), 1.42 (s, 9H, C(CH_3)$_3$), 1.79 (m, 2H, CH$_2$), 1.77 (s, 3H, CH_3), 4.29–4.46 (d, 1H, CHN), 6.00 (s, 1H, C=CH), 8.78 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 12.3 (CH_3), 16.0 (CH_3), 21.5 (CH_3), 25.2 (CH_2), 26.1 (CH_3), 37.6 (CH), 55.9 (CH), 81.8 (C), 104.6 (C), 119.2 (CH), 151.5 (C), 169.9 (C). IR (ν (cm^{-1})): 3430, 1676. HRMS (ESI^+) *m*/*z* calcd for $C_{14}H_{24}N_2O_3Na$, 291.1679 $[M+Na]^+$; found, 291.1676.

*4.2.12. tert-Butyl 2-isopropyl-5-methyl-3-oxo-3,4-dihydropyrazine-1(2H)-carboxylate (**25**).* Brown oil, yield 43%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.85 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 0.92 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 1.42 (s, 9H, C(CH_3)$_3$), 1.47 (m, 1H, CH), 1.76 (s, 3H, CH_3), 4.20–4.36 (d, 1H, CHN), 6.00 (s, 1H,C=CH), 8.55 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 19.3 (CH_3), 21.7 (CH_3), 28.3 (CH_3), 32.6 (CH), 55.6 (CH), 79.9 (C), 102.7 (C), 118.6 (CH), 153.3 (C), 168.9 (C).

*4.2.13. tert-Butyl 2-isobutyl-3-oxo-3,4-dihydropyrazine-1(2H)-carboxylate (**29**).* Brown oil, yield 54%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.98 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 1.01 (d, ^3J=7 Hz, 3H, CH(CH_3)$_2$), 1.50 (s, 9H, C(CH_3)$_3$), 1.66 (m, 3H, CH_2CH), 4.66 (t, ^3J=7 Hz, 1H, CH), 5.70 (t, ^3J=5 Hz, 1H, CH), 6.14 (d, ^3J=5 Hz, 1H, CH), 8.03 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 22.5 (CH_3), 22.6 (CH_3), 24.4 (CH), 28.2 (CH_3), 38.7 (CH_2), 55.7 (CH), 82.0 (C), 108.2 (CH), 108.6 (CH), 152.3 (C), 168.2 (C).

*4.2.14. 3-Isobutyl-5-methyl-3,6-dihydropyrazin-2(1H)-one (**7**).* A solution of **5** (2 g, 6.9 mmol) in dichloromethane (20 mL) was bubbled with HCl gas at room temperature over 4 h. The solvent was removed under reduced pressure to afford a white residue, which was immediately placed under inert atmosphere. Anhydrous diethylether (30 mL), freshly distilled Et_3N (1.4 g, 14 mmol), and activated 4 Å molecular sieves (1 g) were added and the mixture was stirred at room temperature overnight. After filtration, the diethylether was evaporated under reduced pressure to give the target compound as a brown oil (1.15 g, 88%), of sufficient purity for use in the following reaction. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.91 (2d, ^3J=7 Hz, 6H, CH(CH_3)$_2$), 1.45 (m, 1H, CHCH_2CH), 1.68 (m, 1H, CHCH_2CH), 1.91 (m, 1H, CH(CH$_3$)$_2$), 2.08 (s, 3H, CH_3), 3.95 (s, 2H, NHCH_2), 4.06 (m, 1H, CHCO), 8.11 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 22.0 (CH_3), 23.1 (CH_3), 23.9 (CH), 24.6 (CH_3), 42.7 (CH_2), 45.6 (CH_2), 59.1 (CH), 161.4 (C), 171.6 (C). IR (ν (cm^{-1})): 3308, 1710.

*4.2.15. 3-Isopropyl-5-methyl-3,6-dihydropyrazin-2(1H)-one (**8**).* The same procedure as described for **7** was used for cyclisation of **6** and gave **8** as a brown oil in 77% yield. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.81 (d, ^3J=7 Hz, 6H, CH(CH_3)$_2$), 2.05 (s, 3H, CH_3), 2.40 (m, 1H, CH(CH$_3$)$_2$), 3.95 (s, 2H, CH_2), 4.02 (s, 1H, COCHNH), 7.53 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, δ (ppm)): 19.3 (CH_3), 23.8 (CH_3), 32.6 (CH), 46.9 (CH_2), 65.5 (CH), 161.8 (C), 170.5 (C).

*4.2.16. 2-Chloro-3-isobutyl-5-methyl-pyrazine (**9**).* A mixture of **7** (3.78 g, 22.5 mmol), $POCl_3$ (6.7 g, 43.7 mmol), and PCl_5 (5 g, 24 mmol) was stirred at 100 °C for 4 h. Excess $POCl_3$ was removed by distillation under reduced pressure. A saturated aqueous K_2CO_3 solution was added to the residue until pH 8–9. The aqueous layer

was extracted with dichloromethane (3×30 mL). Combined organic layers were washed with brine (50 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give **9** as a brown oil (1.82 g; 44%), of sufficient purity for use in the following reaction. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.92 (d, ^3J=6 Hz, 6H, CH(CH_3)$_2$), 2.26 (m, 1H, C*H*(CH$_3$)$_2$), 2.47 (s, 3H, CC*H*$_3$), 2.74 (d, ^3J=7 Hz, 2H, CC*H*$_2$), 8.00 (s, 1H, C*H*). ^{13}C NMR($CDCl_3$, δ (ppm)): 19.9 (CH_3), 22.2 (CH_3), 28.2 (CH), 43.3 (CH_2), 140.9 (CH), 146.3 (C), 151.2 (C), 154.4 (C). IR (ν (cm^{-1})): 2925, 732.

The same procedure was used for chlorination of **8** and **23**, **24** to give corresponding products **10**, **26**.

*4.2.17. 2-Chloro-3-isopropyl-5-methyl-pyrazine (**10**).* Brown oil, yield 20%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 1.27 (d, ^3J=6 Hz, 6H, CH(C*H*$_3$)$_2$), 2.51 (s, 3H, CC*H*$_3$), 3.55–3.40 (m, 1H, C*H*(CH$_3$)$_2$), 8.02 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.7 (CH_3), 20.8 (CH_3), 31.6 (CH), 140.4 (CH), 145.0 (C), 151.5 (C), 158.8 (C).

*4.2.18. 3-Chloro-2-isobutyl-5-methylpyrazine (**26**).* Brown oil, yield 23%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.95 (d, ^3J=6 Hz, 6H, CH(C*H*$_3$)$_2$), 2.18 (m, 1H, C*H*(CH$_3$)$_2$), 2.51 (s, 3H, CC*H*$_3$), 2.80 (d, ^3J=6 Hz, 2H, C*H*$_2$), 8.29 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.6 (CH_3), 22.4 (CH_3), 28.0 (CH), 43.1 (CH_2), 141.6 (CH), 147.0 (C), 151.0 (C), 152.3 (C).

*4.2.19. 3-Isobutyl-2-methoxy-5-methyl-pyrazine (**11**).* A solution of **9** (1.35 g, 7.3 mmol) in anhydrous methanol (5 mL) was added to a solution of sodium methoxide (freshly prepared by addition of sodium (0.84 g, 36.5 mmol) to 10 mL of anhydrous methanol). The mixture was refluxed for 5 h. The residue obtained after elimination of the methanol by distillation was triturated with diethylether and filtered. The filtrate was evaporated to afford the target compound as a light brown oil (1.13 g, 87%), of 95% purity by NMR. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.91 (d, ^3J=6 Hz, 6H, CH(C*H*$_3$)$_2$), 2.24 (m, 1H, C*H*(CH$_3$)$_2$), 2.41 (s, 3H, C*H*$_3$), 2.63 (d, ^3J=7 Hz, 2H, C*H*$_2$), 3.90 (s, 3H, OC*H*$_3$), 7.74 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.1 (CH_3), 22.2 (CH_3), 27.6 (CH), 41.1 (CH_2), 53.1 (CH_3), 136.4 (CH), 143.6 (C), 146.1 (C), 157.0 (C). IR (ν (cm^{-1})): 2956, 1172. HRMS (ESI$^+$) *m*/*z* calcd for $C_{10}H_{17}N_2O$, 181.1335 [M+H]$^+$; found, 181.1337.

The same procedure was employed for the methoxylation of **10** and **26** into corresponding alkylated methoxypyrazines **12** and **27**.

*4.2.20. 3-Isopropyl-2-methoxy-5-methyl-pyrazine (**12**).* Brown oil, yield 78%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 1.20 (d, ^3J=6 Hz, 6H, CH(C*H*$_3$)$_2$), 2.30 (s, 3H, C*H*$_3$), 3.47 (m, 1H, C*H*(CH$_3$)$_2$), 3.90 (s, 3H, OC*H*$_3$), 7.70 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.7 (*C*H$_3$), 20.8 (CH_3), 32.0 (CH), 53.7 (CH_3), 136.6 (CH), 144.1 (C), 151.9 (C), 159.2 (C).

*4.2.21. 2-Isobutyl-3-methoxy-5-methylpyrazine (**27**).* Brown oil, yield 80%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.95 (d, ^3J=6 Hz, 6H, CH(C*H*$_3$)$_2$), 2.18 (m, 1H, C*H*(CH$_3$)$_2$), 2.39 (s, 3H, C*H*$_3$), 2.65 (d, ^3J=6 Hz, 2H, C*H*$_2$), 3.90 (s, 3H, OC*H*$_3$), 7.90 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.6 (CH_3), 22.4 (CH_3), 27.5 (CH), 40.8 (CH_2), 53.2 (CH_3), 134.3 (CH), 143.8 (C), 147.4 (C), 157.9 (C). HRMS (ESI$^+$) *m*/*z* calcd for $C_{10}H_{17}N_2O$, 181.1335 [M+H]$^+$; found, 181.1337.

4.2.22. 2-Isobutyl-3-methoxypyrazine (IBMP). Brown oil, yield 24%. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.88 (d, ^3J=6 Hz, 3H, CH(C*H*$_3$)$_2$), 0.90 (d, ^3J=6 Hz, 3H, CH(C*H*$_3$)$_2$), 1.95 (m, 1H, C*H*), 2.64 (d, ^3J=6 Hz, 2H, C*H*$_2$), 3.89 (s, 3H, OC*H*$_3$), 7.87 (d, ^3J=2.5 Hz, 1H, C*H*), 7.98 (d, ^3J=2.5 Hz, 1H, C*H*). IR (ν (cm^{-1})): 2956, 1172. HRMS (ESI$^+$) *m*/*z* calcd for $C_{10}H_{17}N_2O$, 181.1335 [M+H]$^+$; found, 181.1332.

*4.2.23. 3-Isobutyl-5-methylpyrazin-2(1H)-one (**13**).* ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.94 (d, ^3J=6 Hz, 6H, CH(C*H*$_3$)$_2$), 2.10 (m, 1H, C*H*), 2.27 (s, 3H, C*H*$_3$), 2.66 (d, ^3J=7 Hz, 2H, C*H*$_2$), 6.95 (s, 1H, C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 21.6 (*C*H$_3$), 22.4 (CH_3), 25.6 (CH), 43.4 (CH_2), 120.6 (CH), 132.6 (C), 156.9 (C), 159.2 (C).

*4.2.24. 5-(3,3-Diethoxy-propyl)-2-isobutyl-3-methoxy-pyrazine (**30**).* To a solution of *t*-BuOK (1.12 g, 10 mmol) in 40 mL of anhydrous THF, *i*-Pr$_2$NH (1.01 g, 10 mmol) was added with a syringe for 15 min, at room temperature. *n*-Butyllithium (2 M/Hexane, 10 mmol, 5 mL) was added at −50 °C then stirred at −30 °C for 30 min. A solution of 2-isobutyl-3-methoxy-5-methylpyrazine **27** (1,6 g, 8.9 mmol) in THF (13 mL) was added at −50 °C. The mixture was stirred at −30 °C for 2 h, and bromoacetaldehyde diethylacetal (2 g, 10 mmol) was added. This solution was stirred for 3 h at −30 °C, left overnight at room temperature then concentrated under reduced pressure. The residue was hydrolyzed with 30 mL of NH_4Cl and extracted with diethylether (3×20 mL). The combined organic extracts were with washed distilled water (2×0 mL) and dried over $MgSO_4$ and concentrated in vacuo to yield **30** as a viscous oil (1.7 g, 70%). This product was used without further purification. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.89 (d, 6H, CH(C*H*$_3$)$_2$), 1.18 (t, ^3J=6 Hz, 6H, OCH$_2$C*H*$_3$), 1.98–2.18 (m 3H, C*H*(CH$_3$)$_2$, C*H*$_2$CH(OCH$_2$CH$_3$)$_2$), 2.62 (s, 2H, NCC*H*$_2$), 2.72 (t, ^3J=6 Hz, 2H, NCC*H*$_2$), 3.64–3.67and 3.51–3.48(2q, ^3J=6 Hz, 4H, OC*H*$_2$CH$_3$), 3.91 (s, 3H, OC*H*$_3$), 4.54 (t, ^3J=6 Hz, 1H, C*H*(OCH$_2$CH$_3$)$_2$), 7.88 (s, 1H, C=C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 15.3 (CH_3), 22.5 (CH_3), 27.5 (CH), 29.6 (CH_2), 32.6 (CH_2), 40.9 (CH_2), 53.1 (CH_3), 61.1 (CH_2), 102.3 (CH), 134.1 (CH), 144.2 (C), 150.4 (C), 158.1 (C). Anal. Calcd for $C_{16}H_{28}N_2O_3$: C, 64.83; H, 9.52; N, 9.45; O, 16.19. Found: C, 65.08; H, 9.37; N, 9.48; O, 16.07. IR (ν (cm^{-1})): 2956, 1171.

*4.2.25. 3-(5-Isobutyl-6-methoxy-pyrazin-2-yl)-propionaldehyde (**31**).* To a stirred solution of trifluoroacetic acid (0.8 mL) in distilled water (0.8 mL), a solution of 5-(3,3-diethoxy-propyl)-2-isobutyl-3-methoxy-pyrazine (1.04 g, 3.5 mmol) in chloroform (3.2 mL) was added at room temperature then warmed to 50 °C for 5 h. Then, the mixture was quenched by the addition of $NaHCO_3$ (powder, up to pH=7.5) and extracted with chloroform (3×20 mL). The combined organic layers were washed with brine (2×10 mL), dried over $MgSO_4$ and evaporated to dryness to give **31** (0.7 g, 90%) as a colorless oil, which was used without further purification. ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.90 (d, 6H, CH(C*H*$_3$)$_2$), 2.04–2.18 (m, ^3J=6 Hz, 1H, C*H*(CH$_3$)$_2$), 2.62 (d, ^3J=6 Hz, 2H, NCC*H*$_2$), 2.86 (t, ^3J=6 Hz, 2H, NCC*H*$_2$), 3.03 (t, ^3J=6 Hz, 2H, OCC*H*$_2$), 3.88 (s, 3H, OC*H*$_3$), 7.92 (s, 1H, C=C*H*), 9.90 (s, 1H, *H*CO). ^{13}C NMR ($CDCl_3$, δ (ppm)): 22.5 (CH_3), 26.7.(CH), 27.5(CH_2), 40.9 (CH_2), 42 (CH_2), 53.3 (CH_3), 134.0 (CH), 144.8 (C), 148.6 (C), 158.1 (C), 201.4 (C). Anal. Calcd for $C_{12}H_{18}N_2O_2$: C, 64.84, H, 8.16, N, 12.60, O, 14.40. Found: C, 65.12, H, 8.21, N, 12.52, O, 14.15. HRMS (ESI$^+$) *m*/*z* calcd for $C_{12}H_{19}N_2O_2$, 223.1441 [M+H]$^+$; found, 223.1440.

*4.2.26. 3-(5-Isobutyl-6-methoxy-pyrazin-2-yl)-propanoic acid (**32**).* A solution of Na_2CO_3 (0.17 g) in water (1.7 mL) was added to 3-(5-isobutyl-6-methoxy-pyrazin-2yl)-propionaldehyde (1.7 g, 7.6 mmol) under stirring at room temperature. The mixture was cooled down to 4 °C and a solution of $KMnO_4$ (1.52 g, 0.96 mmol) in water (30 mL) was added. After stirring for 4 h at 4 °C and 24 h at room temperature, the mixture was filtered off and the aqueous phase was concentrated in vacuo. The solid residue was dissolved in a few drops of water and acidified (pH=5) with citric acid. The resulting solution was extracted with chloroform (3×5 mL), dried over with $MgSO_4$, and concentrated to dryness yielding compound **32** (1.2 g, 66%). ^{1}H NMR ($CDCl_3$, δ (ppm)): 0.89 (br s, 6H, CH(C*H*$_3$)$_2$), 1.98–2.18 (m, 1H, C*H*(CH$_3$)$_2$), 2.62 (br s, 2H, NCC*H*$_2$), 2.78 (br s, 2H, NCC*H*$_2$), 3.01 (br s, 2H, OCC*H*$_2$), 3.87 (s, 3H, OCH$_3$), 7.91(s, 1H, C=C*H*). ^{13}C NMR ($CDCl_3$, δ (ppm)): 22.4 (CH_3), 27.5 (CH), 29.3 (CH_2), 29.6 (CH_2), 40.5 (CH_2), 53.2 (CH_3), 133.4 (CH), 144.2 (C), 149.6 (C), 158.1 (C), 178.2 (C). Anal. Calcd for $C_{12}H_{18}N_2O_3$: C, 60.48, H, 7.61, N, 11.75, O, 20.14. Found: C, 60.73, H, 7.58, N, 11.69, O, 19.98. IR (ν

(cm^{-1})): 3510, 1711, 1177. HRMS (ESI^+) m/z calcd for $C_{12}H_{19}N_2O_3$, 239.1390 $[M+H]^+$; found, 239.1398.

*4.2.27. Methyl 3-(5-isopropyl-3,6-dioxopiperazin-2-yl)propanoate (**34**).* A solution of **33** (15.8 g, 110 mmol), prepared as previously described[30] in dry THF (130 mL), was added dropwise to a stirred solution of L-glutamic acid dimethyl ester hydrochloride[29] (23.2 g, 110 mmol) and triethylamine (24 g, 240 mmol) in dry dichloromethane (120 mL) at −80 °C. The resulting suspension was stirred for 3 h at −70 °C and then allowed to warm to room temperature. The precipitated triethylamine hydrochloride was removed by filtration, and the filtrate was concentrated in vacuo. The crude residue was heated to reflux in toluene (300 mL) for 12 h. Upon cooling, the precipitate was filtered off and dehydrated in vacuo to give a white power (10 g, 36%, mp 175 °C). ^1H NMR (DMSO-d_6, δ (ppm)): 1.22 (d, ^3J=7 Hz, 3H, CH_3), 1.33 (d, ^3J=7 Hz, 3H, CH_3), 2.35 (m, 2H, CH_2), 2.53 (m, 1H, C*H*), 2.80 (m, 2H, CH_2), 3.97 (s, 3H, OCH_3), 4.05 (m, 1H, C*H*), 4.26 (m, 1H, C*H*), 8.48 (s, 1H, N*H*), 8.57 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, δ (ppm)): 18,3 (CH_3), 19.8 (CH_3), 30.1 (CH_2), 30.5 (CH_2), 32.3 (CH), 52.6 (OCH_3), 54.3 (CH), 60.6 (CH), 168.4 (C), 168.9 (C), 174.1 (C).

*4.2.28. Methyl 3-(6-chloro-5-isopropylpyrazin-2-yl)propanoate (**37**).* Compound **34** (2 g, 8.2 mmol) was slowly added to the mixture of $POCl_3$ (5 mL, 54 mmol), and PCl_5 (1 g, 4.8 mmol). The mixture was stirred at 110 °C for an hour. Excess of $POCl_3$ was removed by distillation under reduced pressure. A saturated aqueous K_2CO_3 solution was added to the residue until pH 8–9. The aqueous layer was extracted with diethylether (3×30 mL). Combined organic layers were washed with brine (50 mL), dried over magnesium sulfate, filtered, and concentrated to dryness yielding a mixture of **35**, **36**, and **37**. Compound **35** was separated by flash chromatography (pentane/diethylether 6:4, R_f=0.9).

*4.2.29. Methyl 3-(3,6-dichloro-5-isopropylpyrazin-2-yl)propanoate (**35**).* Oil, yield 25%. ^1H NMR ($CDCl_3$, δ (ppm)): 1.25 (d, ^3J=6.8 Hz, 6H, CH(CH_3)$_2$), 2.80 (t, ^3J=7.3 Hz, 2H, CH_2), 3.18 (t, ^3J=7.3 Hz, 2H, CH_2), 3.47 (septuplet, ^3J=6.8 Hz, 1H, C*H*), 3.68 (s, OCH_3).

The mixture of **36** and **37** (R_f=0.55) recovered was then separated by HPLC on Varian Dynamax Microsorb 100–5 Si column (250×21.4 mm) using the same eluent. The detection was performed at 254 nm. Flow rate was 2 mL/min in the first 5 min, then 0.6 mL/min in the following 35 min. The elution order and retention times were as follows: rt 26.72 min for **36** and rt 31.83 min for **37**.

*4.2.30. Methyl 3-(3-chloro-5-isopropylpyrazin-2-yl)propanoate (**36**).* Brown oil, yield 10%. ^1H NMR ($CDCl_3$, δ (ppm)): 1.26 (d, ^3J=6.8 Hz, 6H, CH(CH_3)$_2$), 2.79 (t, ^3J=6.8 Hz, 2H, CH_2), 3.01 (m, 1H, C*H*), 3.18 (t, ^3J=6.8 Hz, 2H, CH_2), 3.66 (s, OCH_3), 8.24 (s, 1H, *H*ar). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.6 (CH_3), 28.5 (CH_2), 29.9 (CH_2), 30.6 (CH), 51.7 (CH_3), 144.6 (CH), 144.6 (C), 150.4 (C), 157.6 (C), 172.8 (C).

*4.2.31. Methyl 3-(6-chloro-5-isopropylpyrazin-2-yl)propanoate (**37**).* After elimination of solvents, the target compound **37** was obtained as a light brown oil (0,14 g, 7%). ^1H NMR ($CDCl_3$, δ (ppm)): 1.26 (d, ^3J=5.7 Hz, 6H, CH(CH_3)$_2$), 2.79 (t, ^3J=6.8 Hz, 2H, CH_2), 3.06 (t, ^3J=6.8 Hz, 2H, CH_2), 3.47 (m, 1H, C*H*), 3.66 (s, 3H, OCH_3), 8.34 (s, 1H, *H*ar). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.9 (CH_3), 28.9 (CH_2), 29.9 (CH_2), 32.4 (CH), 52.8 (CH_3), 141.6 (CH), 1456.8 (C), 150.5 (C), 159.9 (C),173.1 (C).

*4.2.32. 3-(5-Isopropyl-6-methoxy-pyrazin-2-yl)-propanoic acid (**38**).* A solution of **37** (1.6 g, 6.6 mmol) in anhydrous methanol (5 mL) was added to a solution of sodium methoxide (freshly prepared by careful addition of sodium (0.76 g, 33 mmol) to 10 mL of anhydrous methanol). The mixture was refluxed for 5 h. The crude obtained after elimination of the methanol by distillation was triturated with diethylether and filtered. The filtrate was evaporated to afford crude methyl 3-(5-isopropyl-6-methoxypyrazin-2-yl)propanoate in 90% yield, which was characterized by NMR. ^1H NMR ($CDCl_3$, δ (ppm)): 1.23 (d, ^3J=7 Hz, 6H, 2CH_3), 2.77 (t, ^3J=6 Hz, 2H, CH_2), 3.01 (t, ^3J=6 Hz, 2H, CH_2), 3.30 (m, 1H, C*H*), 3.69 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 7.92 (s, 1H, *H*ar). ^{13}C NMR ($CDCl_3$, δ (ppm)): 20.8 (CH_3), 29.3 (CH_2), 29.5 (CH), 32.8 (CH_2), 51.9 (CH_3), 53.4 (CH_3), 134.1 (CH), 148.8 (C), 149.7 (C), 157.3 (C), 173.6 (C).

The crude obtained after elimination of the methanol was dissolved in water (5 mL), stirred for 15 min at room temperature, then acidified with citric acid up to pH 3. Extraction with diethylether gave **38** as a colorless oil (1.2 g, 81% from **37**). ^1H NMR ($CDCl_3$, δ (ppm)): 1.21 (d, ^3J=7,5 Hz, 6H, 2CH_3), 2.27 (t, ^3J=6 Hz, 2H, CH_2), 3.01 (t, ^3J=7.1 Hz, 2H, CH_2), 3.29 (m, 1H, C*H*), 3.90 (s, 3H, OCH_3), 7.95 (s, 1H, *H*ar). ^{13}C NMR ($CDCl_3$, δ(ppm)): 20.8 (CH_3), 29.1 (CH_2), 29.6 (CH), 32.8 (CH_2), 53.7 (CH_3), 133.9 (CH), 149.1 (C), 149.8 (C), 157.6 (C), 178.6 (C). IR (ν (cm^{-1})): 3408, 1713. HRMS (ESI^+) m/z calcd for $C_{11}H_{17}N_2O_3$, 225.1233 $[M+H]^+$; found, 225.1236.

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