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1,1-Diphenyl-2-methylsulfinyl ethanol: a model compound to study the reactivity towards CNBr of a photoadduct of methionine on $C_\gamma H_2$

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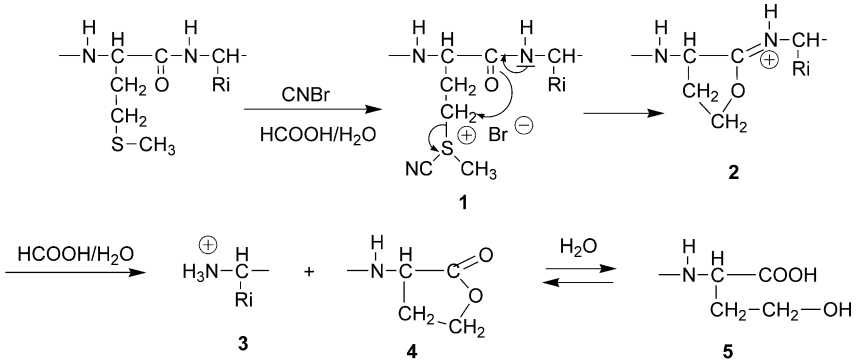
Abstract—The photoadduct formed by photolysis of the [Bapa^0, (*p*Bzl)Phe^5, Met$(O_2)^{11}$]SP/NK-1 complex localised within the T^{173}MP^{175} domain of the NK-1 receptor cannot be cleaved by CNBr on the C-side of methionine; an unusual rearrangement of the intermediate sulfonium instead occurred. The reactivity of 1,1-diphenyl-2-methylsulfinyl ethanol **10** towards CNBr treatment and the stability of the 1,1-diphenyl oxirane **14** were analysed by NMR and mass spectrometry. 1,1-Diphenyl ethylene **13** can be formed from epoxide **14** even in slightly acidic conditions and during positive DCI/NH_3 mass spectrometry analysis. Altogether, these results suggest that if a covalent linkage between the [Bapa^0, (*p*Bzl)Phe^5, Met$(O_2)^{11}$] and the NK-1 receptor occurred on the $C_\gamma H_2$ of methionine-174, CNBr treatment will lead to an epoxide/ketone and an ethylenic compound. © 2002 Published by Elsevier Science Ltd.

The chemical cleavage of proteins by cyanogen bromide is widely used as this treatment in acidic medium ($HCOOH/H_2O$) leads to disruption of a specific amide bond only after a methionine residue. The iminolactone **2**, obtained by intramolecular elimination of the sulfonium **1**, is subsequently hydrolysed into homoserine lactone **4** in equilibrium with homoserine **5** (Scheme 1).[1] We have used such a strategy to establish that the specific covalent linkage obtained with a substance P (SP: H-RPKPQQFFGLM-NH_2) photoreactive analogue, [Bapa^0, (*p*Bzl)Phe^8]SP, localised within the T^{173}MPSR^{177} domain of the specific NK-1 receptor, has occurred on the methyl of methionine-174 (Scheme 2).[2–4] With the photoreactive analogue of SP modified at position 5, [Bapa^0, (*p*Bzl)Phe^5, Met$(O_2)^{11}$]SP,[5] a covalent linkage was shown to be in the same T^{173}MPSR^{177} domain of the NK-1 receptor after enzymatic digestions of the NK-1/ligand complex. Further digestion of this pentapeptide linked to the SP analogue restricted the domain to the tripeptide T^{173}MP^{175}.[6]



Scheme 1.

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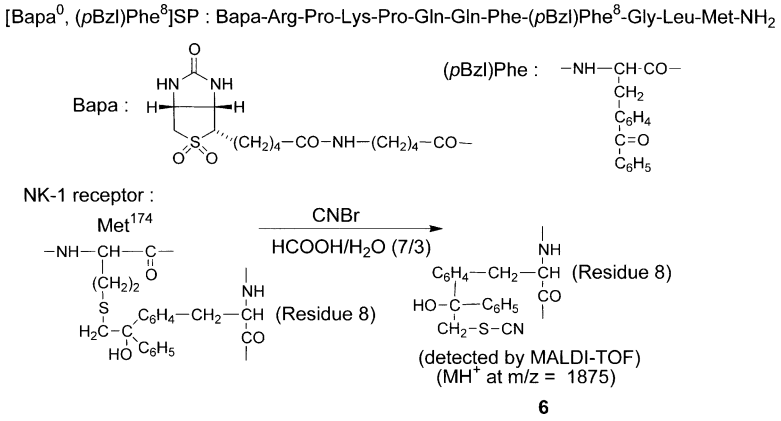
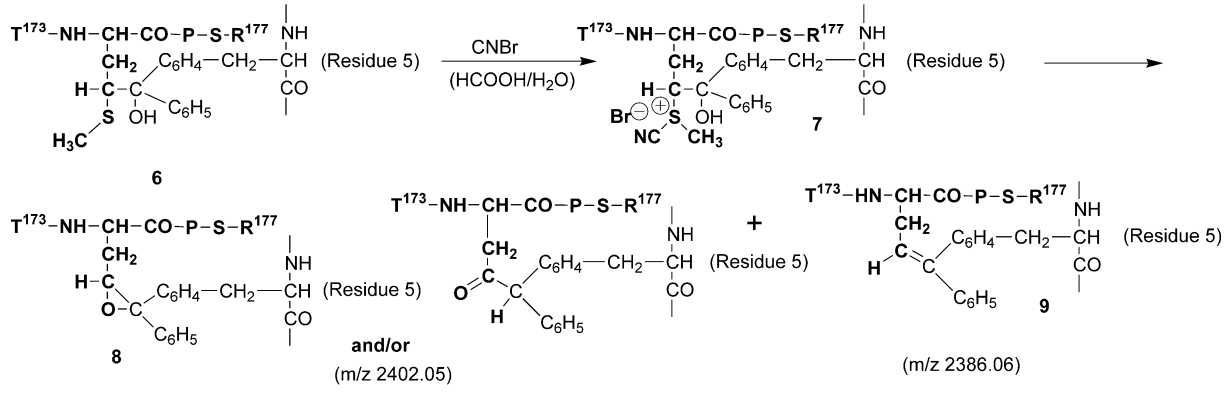


Scheme 2.

Cyanogen bromide treatment of this $T^{173}MPSR^{177}$ fragment (MH^+ at m/z 590.19 measured, 590.29 expected) specifically modified by [$Bapa^0$, (pBzl)Phe^5, $Met(O_2)^{11}$]SP (MH^+ at m/z 1859.90) led to the detection by MALDI-TOF mass spectrometry of two specific signals at m/z 2386.06 and m/z 2402.05. The 'usual' CNBr cleavage detailed in Scheme 1 did not occur. We propose that the insertion of [$Bapa^0$, (pBzl)Phe^5, $Met(O_2)^{11}$]SP into the NK-1 receptor has occurred with this photolabelled analogue on the $C_\gamma H_2$ of the methionine-174 side chain (Scheme 3). Deseke et al. have recently shown that *N*-acetylmethionine methyl ester reacted selectively with benzophenone at the carbon atom adjacent to sulfur and the $C_\gamma H_2$ addition product is predominantly formed.

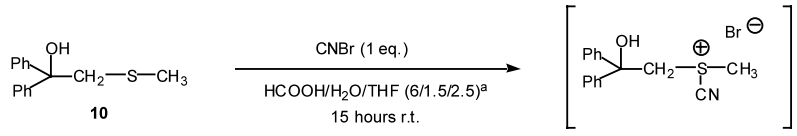
The steric hindrance at C_γ may explain the failure of the 'usual' CNBr cleavage, instead, CNBr treatment led to the sulfonium **7** which can evolve to the epoxide (or ketone) **8** (MH^+ at m/z 2402.05) and the ethylenic compound **9** (MH^+ at m/z 2386.06). Epoxide formation may come from an intramolecular substitution and the ketone from transposition of this epoxide. However, the presence of the ethylenic compound **9** cannot be explained by a concerted elimination (Wittig type) as this mechanism prevalent with phosphonium has never been evidenced with sulfonium derivatives.[7]

To support this hypothesis the model compound **10** mimicking the reaction product **6** of the *para*-benzoyl-L-phenylalanine SP analogue linked to the $C_\gamma H_2$ of the methionine has been prepared from benzophenone and di-methylsulfoxide.[8,9] 1,1-Diphenyl-2-methylsulfinyl ethanol **10** has been reacted with CNBr (1 equiv.) in $HCOOH/H_2O/THF$ (6/1.5/2.5). After extraction (usual work-up), the crude mixture has been analysed by NMR and DCI/NH_3 mass spectrometry; all the data are reported in Table 1. After 15 hours (conditions used for the SP/NK-1 complex), the starting product **10** has almost completely disappeared (4% remaining) and no intermediate sulfonium was detected. The major compounds detected by NMR are the dehydration product of the starting material **11** and the demethylation product **12** of the sulfonium intermediate by a nucleophile, Br^- for example. Besides **11** and **12**, the ethylenic compound **13** (3%) and trace amount ($\leq 1\%$) of epoxide **14** were also found. All these products were also detected by DCI/NH_3 mass spectrometry; the starting material **10** yields the dehydration product **11**, the ethylenic compound **13** leading to the major signal.



Scheme 3.

Table 1. NMR and mass spectrometry analysis of the reaction products formed by CNBr treatment of 1,1-diphenyl-2-methylsulfinyl ethanol **10**



	10	**11**	**12**	**13**	**14**	**15**
NMR[b]	4 %	31 %	62 %	3 %	≤ 1 %	n.d.
DCI/NH_3[b] Mass spectrometry	n.d.[c]	MH^+ 227 (0.5) MNH_4^+ 244 (0.1)	MH^+ 256 (0.3) MNH_4^+ 273 (0.5) (also observed the dehydration product at MH^+ 238 ; 0.1)	MH^+ 181[d] (1)	MH^+ 197[e] (0.05) MNH_4^+ 214[e] (0.1)	

a) THF was necessary to complete dissolution of **10**, otherwise solvent conditions, temperature and incubation time were the same as those used for the experiments performed with the photolabelled NK-1 receptor. ^1H- and ^{13}C-chemical shifts of all compounds found in the crude mixtures were assigned using HSQC and HMBC sequences and are listed with the following internal references, for ^{13}C : $CDCl_3$ at 77.1 ppm and for ^1H : TMS at 0 ppm. 1,1-diphenyl-2-methylsulfinyl ethanol, **10** : CH_3 (17.6, 1.99), CH_2 (48.5, 3.44), $C(Ph)_2OH$ (75.5) ; 2,2-diphenyl-thiomethyl ethylene, **11** : CH_3 (18.1, 2.38), CH (127.6, 6.55), $C(Ph)_2$ (138.5) ; 1,1-diphenyl-2-thiocyano ethanol, **12** : CN (112.8), CH_2 (47.3, 3.92), $C(Ph)_2OH$ (77.8) ; 1,1-diphenylethylene, **13** : CH_2 (114.4, 5.46), $C(Ph)_2$ (138.5) ; 1,1-diphenyl oxirane, **14** : CH_2 (69.5, 3.28), $C(Ph)_2O$ (78.6) ; 2,2-diphenylethanal, **15** ; COH (198.6, 9.95 (d)), $C(Ph)_2H$ (64.2, 4.89 (d)).

b) Analysis of the crude products obtained after extraction of the reaction mixture.

c) When **10** was analysed by DCI/NH_3 mass spectrometry, only the dehydration product **11** was observed. n.d. : Not detected

d) The olefin **13** corresponds to the most intense signal and will be taken as the base peak (1). Intensity is not a criterium of concentration but reflects the ionisation capability of the molecule, as evidenced by a co-injection of a 1/1 mixture of pure compounds **13** and **14**.

e) The epoxide **14** and aldehyde **15** have identical MH^+/MNH_4^+, however only traces of epoxide **15** were evidenced by NMR, the aldehyde was not present in the crude mixture.

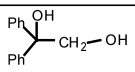
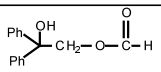
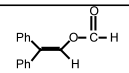
So, this first experiment proved that both an epoxide and an ethylenic compound can be formed by reacting with CNBr a model compound mimicking the benzophenone-$C_\gamma H_2$ methionine adduct **6**.

Concerning the mechanism of formation of the epoxide **14**, an intramolecular nucleophilic substitution of the sulfonium intermediate should be the driving force, even in acidic conditions. Did the ethylenic compound come from a rearrangement of the sulfonium (Wittig type) or from the epoxide **14**? To answer this question the reactivity/stability of the epoxide **14** was then studied. Epoxide **14** was prepared by reacting benzophenone with trimethylsulfonium iodide and sodium hydride.[10] Mass spectrometry analysis (DCI/NH_3) revealed that this pure epoxide **14** (NMR) yields three protonated molecules: the epoxide **14** (both forms MH^+ and MNH_4^+) and the ethylenic compounds **13** (MH^+ at m/z 181) (Table 2). Thus, this experiment proved that the ethylenic compound 1,1-diphenyl ethylene **13** can be formed by pyrolysis from the epoxide **14** during mass spectrometry analysis.

This ethylenic compound **13** can also be formed in solution when epoxide **14** is reacted with CNBr in $HCOOH/H_2O/THF$ (Table 2). With the conditions used for the photolabelling studies ($HCOOH/H_2O$/THF), NMR analysis of the crude mixture, after extraction and usual work-up, demonstrated that epoxide **14** was totally transformed into aldehyde **15** ($\leq 5\%$), diol **16** (35%) and an addition product **17** (60%) which must come from an opening of the epoxide by formic acid. Mass spectrometry analysis confirmed the presence of these three species **15** (MH^+, MNH_4^+), **16** (MNH_4^+), **17** (MNH_4^+) and its dehydrated form **18** (MH^+, MNH_4^+). The presence of MH^+ at m/z 183 and MNH_4^+ at m/z 200 in the crude mixture suggested that the ethylenic compound **13** must have been formed in solution and in situ reduced by formic acid into 1,1-diphenyl ethane **19** (MH^+ at m/z 183), which was not identified by NMR representing probably less than 1% in the crude mixture.

This experiment and the relative stability of the epoxide **14** during flash chromatography led us to finally study its stability in slightly acidic conditions. So, a solution of epoxide **14** let in $CDCl_3$ in the presence of SiO_2 at room temperature, was analysed by NMR and mass spectrometry. After 36 hours more than 50% of the epoxide has been converted (NMR analysis) into aldehyde **15** (25%) and diol **16** (25%); the ethylenic compound **13** was also present in solution (2%). After 1 week standing, epoxide **14** has completely been transformed into aldehyde **15** (47%), diol **16** (47%), the amount of 1,1-diphenylethylene **13** being always around 2%. Besides **15**, **16** and **13**, the addition product of formic acid on the epoxide **17** was also identified (2%); its formation can be explained by traces of phosgene formed in $CDCl_3$ and subsequent hydrolysis into formic acid.[11] Finally, after 1 month standing, mass spectrometry analysis showed that **15**, **16**, **17** and its dehydrated form **18** were present; the ethylenic compound **13** has

Table 2. Reactivity and stability of 1,1-diphenyl oxirane **14**

	 **14**	 **15**	 **16**	 **13**	 **17**	 **18**	 **19**
				Epoxide **14**			
NMR[a,b]	100 %	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DCI/NH_3[b]	MH^+ 197[b] (1) MNH_4^+ 214[b] (1)	c)	n.d.	MH^+ 181 (0.2)	n.d.	n.d.	n.d.
			Epoxide **14**, CNBr (1 eq.) in HCOOH/H_2O/THF (6/1.5/2.5), 15 hours [b]				
NMR	n.d.	≤ 5 %	35 %	n.d.	60 %	n.d.	n.d.
DCI/NH_3[b]	c)	MH^+ 197 (0.4) MNH_4^+ 214 (0.4)	MNH_4^+ 232 (0.1)	n.d	MNH_4^+ 260 (0.6)	MH^+ 225[b] (1) MNH_4^+ 242 (0.2)	MH^+ 183 (0.5) MNH_4^+ 200 (0.1)
			Epoxide **14**, $SiO_2/CDCl_3$				
NMR (36 hours)	48 %	25 %	25 %	2 %	n.d.	n.d.	n.d.
NMR (one week)	n.d.	47 %	47 %	2 %	2 %	2 %	n.d.
DCI/NH_3 (one month)	n.d.	MH^+ 197 (0.8) MNH_4^+ 214[b] (1)	MNH_4^+ 232 (0.3)	n.d.	MNH_4^+ 260 (0.05)	MH^+ 225 (0.1) MNH_4^+ 242[c] (0.02)	MH^+ 183 (0.3) MNH_4^+ 200 (0.06)

a) ^1H- and ^{13}C-chemical shifts of all compounds found in the crude mixtures were assigned using HSQC and HMBC sequences and are listed with the following internal references, for ^{13}C : $CDCl_3$ at 77.1 ppm and for ^1H : TMS at 0 ppm. 1,1-diphenylethanediol, **16** : CH_2 (69.5, 4.17), $C(Ph)_2OH$ (78.6) ; 2-hydroxyl-1,1-diphenyl ethyl formate, **17** : OCOH (8.05), CH_2 (69.2, 4.77), $C(Ph)_2OH$ (77.1).

b) Analysis of the crude products obtained after extraction of the reaction mixture, the most intense signal of each spectra was taken as the base peak (1).

c) Epoxide **14** and aldehyde **15** have identical MH^+/MNH_4^+, NMR analysis supported the presence of only aldehyde **15**. n.d. : Not detected.

been reduced by formic acid into 1,1-diphenylethane **19**.

These experiments were designed to determine the reactivity towards CNBr treatment of the addition product **6** described in Scheme 2. Altogether, these experiments done with a model compound such as **10** demonstrate that (i) the epoxide/aldehyde, **14**/**15**, can be formed from the model compound **10** when reacted with CNBr in aqueous formic acid, (ii) epoxide **14** yields the ethylenic compound **13** by pyrolysis during mass spectrometry analysis and (iii) the epoxide **14** can also yield the ethylenic compound **13** even in slightly acidic medium. The proportions of the compounds determined by NMR and mass spectrometry cannot be compared. Indeed, relative intensities in mass spectrometry only reflect the capability of one molecule versus another to be ionised and these results showed that this potency is highly increased with 1,1-diphenylethylene **13** compared to epoxide/aldehyde **14**/**15**.

These experiments with a model compound constitute an indirect but convincing proof for the postulated formation of a covalent photoadduct between [$Bapa^0$, (pBzl)Phe^5, $Met(O_2)^{11}$]SP and the $C_\gamma H_2$ of methionine-174 within the $T^{173}MP^{175}$ domain of the human NK-1 receptor.

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