A rapid and convenient method for specific ^{11}C-labelling of synthetic polypeptides containing methionine

B. Långström, S. Sjöberg and U. Ragnarsson*
Department of Organic Chemistry, Institute of Chemistry,
University of Uppsala, Box 531, S-751 21 Uppsala, SWEDEN
*Institute of Biochemistry, Biomedical Center,
University of Uppsala, Box 576, S-751 23 Uppsala, SWEDEN

INTRODUCTION

An increasing interest in the short-lived positron-emitting radionuclides like ^{11}C ($t_{\frac{1}{2}}$ = 20.4 min) has been an incitement for developing rapid synthetic routes for labelling low molecular weight compounds of biomedical interest.[1]

However, there are only a few examples reported in the literature dealing with the labelling of polypeptides or proteins with short-lived radionuclides.

SUMMARY

^{11}C-labelling of methionine residues in a synthetic peptide via the preparation of the corresponding protected, pure homocysteine peptide has been investigated. Complete deprotection of the peptide and specific methylation of the homocysteine residue can be performed in one step in liquid ammonia. As a first application of this method the synthesis of the tripeptide, Z-Gly-L-Hcy(Bzl)-Gly-O-Bzl, and its conversion to Gly-Met-Gly and the corresponding labelled Gly-([^{11}C]-methyl)-Met-Gly, is reported. Starting with the protected peptide the labelling was performed in 20 ± 5 min (starting with $^{11}CO_2$), yielding the labelled peptide in 92 ± 5 % radiochemical yield. Analyses and preparative LC can be performed within 6 min.

Key Words: ^{11}C-peptides, labelled peptides, labelled methionine peptides, peptide synthesis.

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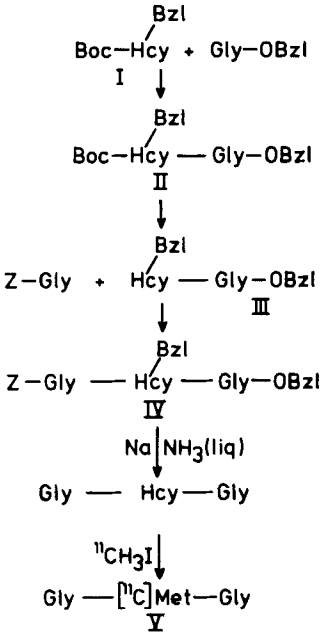
An example is the unspecific labelling of α-chymotrypsin[2] using ^{11}C generated by proton irradiation of water solutions.[3] Other examples are the specific labelling of fibrinogen,[4] albumin[4] and the ovine luteinizing hormone,[5] yielding slightly modified proteins.

In the synthesis of [^{11}C]-methyl- L -methionine,[6] the alkylation was performed with $^{11}CH_3I$ on the sulfide anion of homocysteine generated from L -S-benzylhomocysteine with sodium in liquid ammonia. In order to investigate if similar reaction conditions could be used for the specific labelling of methionine-containing peptides, a simple peptide was synthesized. The tripeptide Z-Gly-L-Hcy(Bzl)-Gly-O-Bzl was selected, being a model for neurohormones such as methionine-enkephaline[7a] and substance P.[7b]

SYNTHETIC PATHWAYS

The Boc derivative of S-benzylhomocysteine (I) was prepared by analogy with corresponding proline[8] derivative and coupled with the benzyl ester of glycine (Scheme 1). Dicyclohexylcarbodiimide was used in the formation of the peptide bonds. The Boc protecting group was removed with trifluoroacetic acid. The crude dipeptide L-Hcy(Bzl)-Gly-O-Bzl (III), obtained after liberation from its trifluoroacetic acid salt, was directly coupled to benzyloxycarbonylglycine yielding the tripeptide (IV). In the alkylation experiments all the protecting groups were removed by reaction with sodium in liquid ammonia[9] after which methyl iodide was added to the reaction mixture.



Scheme 1.

RESULTS

The tripeptide (IV) was obtained in 30 % chemical yield, but no attempts have been made at this stage to optimize this or other synthetic steps. The peptide was characterized by NMR and elemental analysis. The peak assignments in the ^{13}C-NMR analysis were made with off-resonance decoupling and shift values from literature.[10] In the alkylation experiments the protected peptide (IV) was dissolved in liquid ammonia and sodium metal added. Preliminary investigations with labelled and unlabelled methyl iodide showed that the amount of sodium added to the reaction mixture was critical to the outcome of the reaction. If sodium was added so that the blue colour lasted for approximately 30 seconds or more, an increasing yield of a dipeptide

byproduct, Gly-Met or Met-Gly, was obtained, as indicated by preparative LC and conventional amino acid analysis after hydrolysis of the separated product. This is consistent with other observations that sodium in liquid ammonia can in some cases cleave peptide bonds.[11]

Considering these results, the labelling experiments were performed by adding the sodium to the solution so that the blue colour only lasted for a few seconds after which only one labelled peptide, Gly-[^{11}C]-Met-Gly was obtained.

The labelled methyl iodide used in the experiments was produced from $^{11}CO_2$ according to the procedure earlier described[12] and trapped directly in the liquid ammonia solution at -78 °C. After a few minutes the ammonia was removed and the solid residue dissolved in water. The solution was pH adjusted to 7 and millipore filtered. Analysis with LC showed a 92 ± 5 % radiochemical yield of Gly-[^{11}C]Met-Gly. The total time used for the synthesis from releasing $^{11}CO_2$ from a trap of molecular sieves was 20 ± 5 min (4-7 min for $^{11}CH_3I$ and 11-18 min for the alkylation). So far no efforts have been undertaken to shorten the time of the peptide labelling.

In two labelling experiments carrier methyl iodide was added shortly after the trapping of the $^{11}CH_3I$. Separations of the products by preparative LC and amino acid analysis showed a glycine/methionine ratio of 2.05 ± 0.02/0.95 ± 0.02 for the labelled product.

The method presented here should be generally applicable for labelling peptides which can be synthesized containing S-protected homocysteine or homocysteine itself. Further investigations are now in progress for labelling neurohormones.

EXPERIMENTAL

General. NMR spectra were recorded on a JEOL FX-100. The compounds were identified by ^{13}C-NMR and elemental analyses. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. LC was performed on a Waters system, pump 6000 equipped with a UV 4100 in series with a NaI (Tl) crystal for radioactivity measurements. The preparative LC was made on a C-18 Bondapak column (Waters) and analyses on a Partisil 10-ODS column (Reeve Angel).

The ^{11}C was produced using a gas target with nitrogen (AGA-SR) by the ^{14}N(p, α) ^{11}C reaction[13] on the tandem Van de Graaff accelerator at the University of Uppsala. The ^{11}C was obtained as $^{11}CO_2$ in a trap containing 5 A molecular sieves.

N-t-Butyloxycarbonyl-S-benzyl-L-homocysteine (Boc-L-Hcy(Bzl))(I)

A mixture of 11.2 g (0.05 mole) of L-S-benzylhomocysteine, 11.5 g (0.10 mole) tetramethylguanidine and 19.4 g (0.05 mole) of t-butylphenylcarbonate in 50 mL of dry dimethylsulfoxide was stirred at room temperature for 60 h. The solution was then poured into 150 mL of 1 M sodium hydrogen carbonate ($NaHCO_3$) and 100 g of ice. The mixture was extracted with 3 x 100 mL of ether. The water phase was acidified with potassium hydrogen sulfate ($KHSO_4$) to pH 3 and the liberated Boc-Hcy(Bzl) was extracted with 3 x 150 mL of ether. The combined ether phase was washed with water and brine, dried over sodium sulfate and finally evaporated. The oily residue, 15.4 g, was dissolved in ether. The crude acid (I) was purified by crystallization of its dicyclohexylamine salt, m.p. 133-134.5 ^{o}C. The acid was liberated from the salt by ether extraction of a solution acidified to pH 3. The acid was obtained in a 8.0 g (49 %)

yield, m.p. 101-102 °C, $[\alpha]_{578}^{25.0}$ = -4.4° (c = 2.9 in ethanol). (Found/calculated: C, 58.82/59.07; H, 7.11/7.07; N, 4.23/4.31 and S, 9.35/9.85).

^{13}C-NMR (100 MHz, $CDCl_3$, δ relative TMS): 26.8 (Hcy-C_4); 28.1 (Boc-CH_3); 31.9 (Hcy-C_3); 36.1 (-CH_2-S); 52.6 (Hcy-C_2); 81.0 (Boc-C); 126.8 (Phenyl-C_4); 128.3 (Phenyl-$C_{3,5}$); 128.6 (Phenyl-$C_{2,6}$); 137.8 (Phenyl-C_1); 156.0 (-NH-CO_2-); 176.2 (-CO_2H).

N-t-Butyloxycarbonyl-S-benzyl-L-homocysteine-glycine benzyl ester (Boc-L-Hcy(Bzl)-Gly-O-Bzl) (II)

Boc-Hcy(Bzl) (I), 4.87 g (0.015 mole), was mixed with 2.75 g (0.0166 mole) of the benzylester of glycine and 3.09 g (0.015 mole) of dicyclohexylcarbodiimide (DCC) in 50 mL of dichloromethane and was stirred at 0 °C for 1 h. The temperature was then raised to 25 °C and the mixture stirred for another hour. The precipitated urea was filtered off and the filtrate was evaporated after which the residue was dissolved in ether. The organic phase was washed with $KHSO_4$ (pH 3) and with $NaHCO_3$ (pH 8). The ether was then dried and evaporated, yielding 7.0 g of a crude dipeptide (II), obtained as an oil, which was used in the next step without further purification. A part of this oil crystallized on standing. The pure dipeptide II was obtained after recrystallization in ethylacetate and petroleum ether, m.p. 38-40 °C, $[\alpha]_{578}^{25.0}$ = -10.6° (c = 2.7 in ethanol).

(Found/calculated: C, 63.73/63.56, H, 7.18/6.78, N, 5.58/5.93, S, 6.06/6.77).

^{13}C-NMR (100 MHz, $CDCl_3$, δ relative TMS): 27.2 (Hcy-C_4); 28.3 (Boc-CH_3); 31.9 (Hcy-C_3); 35.9 (-CH_2-S); 41.2 (Gly-C_2); 53.2

(Hcy-C_2); 67.0 (-CH_2-O); 80.0 (Boc-C); 126.7, 128.1, 128.2, 128.7 (Phenyl-$C_{4,4',3,3',5,5',2,2',6,6'}$); 134.9, 138.0 (Phenyl-$C_{1',1}$); 155.3 (-NH-$CO_2$-); 169.1, 171.7 (-CONH).

N-Benzyloxycarbonylglycine-S-benzyl-L-homocysteine-glycine-benzylester (Z-Gly-L-Hcy(Bzl)-Gly-O-Bzl) (IV)

All the crude dipeptide Boc-Hcy(Bzl)-Gly-O-Bzl (II) was dissolved in 12 mL of trifluoroacetic acid and 2 mL of dimethylsulfide. After stirring for 30 min at room temperature the TFA was evaporated. After liberation from the TFA-salt the residue was mixed with 3.0 g (0.014 mole) of benzyloxycarbonylglycine and 1.73 g (0.0084 mole) DCC in dichloromethane and ethylacetate. After 60 min at 0 °C the urea was removed by filtration and the tripeptide (IV) was isolated as described for (II). The residue crystallized on standing, yielding 2.8 g (30 %) of crude IV. Recrystallization from ethylacetate and petroleum ether gave the pure peptide, m.p. 133-135 °C, $[\alpha]_{578}^{25.0} = -11.2^{o}$ (c = 0.73 in ethanol). (Found/calculated: C, 63.79/63.94; H, 6.05/5.86; N, 7.48/7.46 and S, 5.55/5.68).

^{13}C-NMR (100 MHz, $CDCl_3$, δ relative TMS): 27.1 (Hcy-C_4); 31.5 (Hcy-C_3); 35.8 (-CH_2-S); 41.8 (Gly-C_2); 44.2 (Gly-C_2); 52.0 (Hcy-C_2); 67.1 (-CH_2-O); 126.9, 127.8, 128.4, 128.8. (Phenyl-$C_{4,4',4'',3,3',3'',5,5',5'',2,2',2'',6,6',6''}$); 135.0, 136.0, 138.0. (Phenyl-$C_{1',1'',1}$); 156.5 (-NH-CO_2-); 169.2, 171.2 (-CONH-).

Glycine-L-([^{11}C]-methyl)methionine-glycine

(Gly-L-[$^{11}CH_3$]-Met-Gly) (V)

Shortly before the preparation of the ^{11}C-methyl iodide, 0.020 g (3.6×10^{-5} mole) of Z-Gly-L-Hcy(Bzl)-Gly-O-Bzl was dissolved in 2 mL of dried liquid ammonia and sodium was added until the blue colour lasted for a few seconds. The $^{11}CH_3I$ was trapped in the reaction mixture at -78 ^{o}C after which the reaction vial was removed from the cooling bath and allowed to stand at room temperature for 5 min. The ammonia was evaporated and the solid residue dissolved in 2 mL of water. The solution was adjusted to pH 7. Sterile filtration gave a clear solution which was analyzed with LC on a reverse phase column with methanol/water (4/96).

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