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HOMOCYSTEINE THIOLACTONE AS PRECURSOR OF METHIONINE AMIDE : APPLICATION TO THE MODIFICATION OF PEPTIDES OF THE TACHYKININ FAMILY

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Abstract

Two pathways for the substitution of a tert-butyl group from (S-tert-butyl)-homocysteine by an alkyl residue are described. Pathway B via homocysteine thiolactone, can be used to modify any peptide containing a C-terminal methionine amide.

The neuropeptide Substance P, (SP), is a member of the tachykinin family, a group of peptides which share, among other common features, a C-terminal methionine amide (1). Recently, two other tachykinins, NKA and NKB, (2-5) have been found in the central nervous system and the functions of these peptides have to be investigated. This prompted us to develop a general methodology for transforming the methionine residue into a large variety of analogues modified on sulfur or/and on the terminal carboxamide, in order to study structure-activity relationships or to obtain labelled ligands.

Substance P (SP) : Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

Neurokinin α (NKA) : His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2

Neurokinin β (NKB) : Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

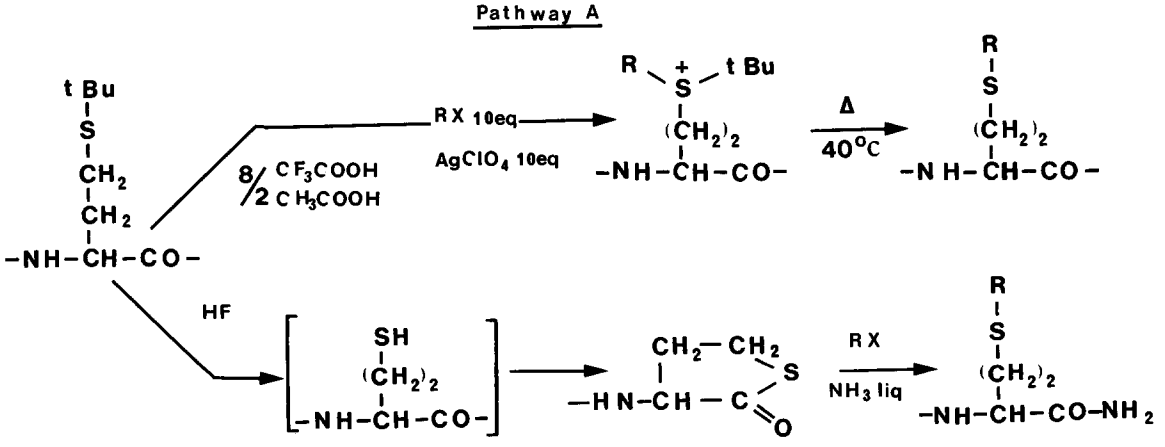
We described recently the two-step conversion of methionine into (S-tert-butyl)-homocysteine (6). In the present report, we propose two pathways for the substitution of

the tert-butyl group by an alkyl residue. In this study conducted to test the method, the alkylating agent was either methyl iodide or trideuteriomethyl p-toluene sulfonate.

Pathway A takes advantage of the easy thermal decomposition of tert-butyl sulfonium salts. It relies however on the possibility of obtaining the dissymmetric sulfonium salts.

For instance, the alkylation of (S-tert-butyl)-homocysteine by ICH_3 (50 equiv) in H_2O, at room temperature for 2 hours, led to the dimethyl sulfonium salt. The dissymmetric sulfonium could be obtained using 10 equiv. of the $ICH_3/AgClO_4$ complex at lower temperature (5°C) in CF_3COOH/CH_3COOH (2:8). After two hours, where the excess of ICH_3 has been consumed further addition of H_2O and warming for 2 hours at 40°C yielded methionine with an excellent yield (90%).

The same conditions applied to {S-tert-butyl-Hcy[11]} SP induced the precipitation of the peptide, and by increasing the concentration of CF_3COOH, the precipitation of $AgClO_4$. This can be overcome by using $AgBF_4$, more soluble in trifluoroacetic acid. However, in less than 5 min at 5°C in CF_3COOH we detected only the dimethyl sulfonium salt coming from the alkylation of the intermediate sulfide by either ICH_3 or the dissymmetric sulfonium



Scheme 1

salt. (7). As it has been observed that the electrophilicity of these salts is very depen-

dent on the various counter-ions and solvents (8), we checked a range of conditions. In the best that we found {CF_3COOH/CH_3SO_3H (10:1) with 10 equiv. of $ICH_3/AgClO_4$} we obtained a 4:1 ratio of symmetric and dissymmetric sulfoniums. Thus, we had to use another route.

Pathway B : The intermediate thiol {Hcy^{11}} SP generated by HF cleavage of { S-tert-butyl-Hcy^{11} } SP from the methylbenzhydrylamine polymer (MBHA) cannot be isolated and cyclises into the corresponding thiolactone peptide. We have previously attributed the thiol structure to the intermediate on the basis of amino-acid analysis and of its alkylation in liquid ammonia by methyl chloride (6). In fact, the isolated {Hcy^{11}} SP thiolactone intermediate was opened in liquid ammonia yielding the C-terminal amide and the thiol function thus generated was alkylated in situ by CH_3Cl.

The thiolactones of methionine, SP, NKA and NKB have been obtained from the corresponding StBu precursors. The peptides have been synthesized by solid-phase, the first residue being always N-α-Boc-S(tert-butyl)-homocysteine, linked to a MBHA polymer. After cleavage by HF and usual work-up, the crude peptides have been purified by classical techniques using either acidic or neutral eluants. The thiolactone function has been characterized by 250 MHz ^{1}H-NMR (two multiplets 3.25-3.17 ppm and 2.09 ppm respectively for the γ-CH_2 and β-CH_2 of the thiolactone ring) and by U.V. (the UV difference spectra between the thiolactone and the corresponding C-terminal methionine amide peptides present the same maximum at 238 nm (ε=3600)). The structure was confirmed by FAB Mass Spectrometry: {Hcy^{11}} SP thiolactone (MH^+, m/z 1316) ; { Hcy^{10}}NKA thiolactone (MH^+, m/z 1102) ; { Hcy^{10}} NKB thiolactone (MH^+, m/z 1181).

The two methods we have described are complementary. Pathway A, when possible, is preferable, because of simpler experimental conditions. Pathway B is more general. The homocysteine thiolactone at the C-terminal position of a peptide is a stable precursor of methionine amide residue. It can be used for labelling a C-terminal methionine amide with a radioactive group and for the obtention of S modified analogs. It can also be opened by different nucleophiles to obtain C-terminal modified analogs.

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