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AMINO ACID SURROGATES: AN INDIRECT METHOD FOR THE SYNTHESIS OF PEPTIDES CONTAINING THE THIOASPARAGINYL RESIDUE

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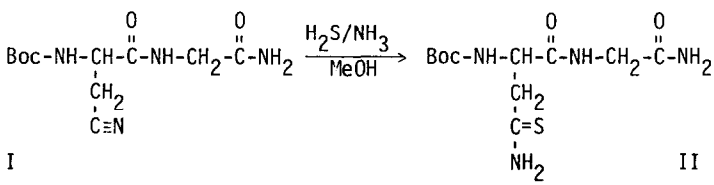
ABSTRACT: The protected pentapeptide, Boc-Tan-Cys(PMB)-Pro-Leu-GlyNH_2[†] and related thioasparaginyl compounds were prepared by an indirect method from their corresponding β-cyanoalanyl precursors by treatment with H_2S/NH_3. The optical purities of thioasparagine (Tan) derivatives were evaluated by two different approaches.

Previously three separate groups have successfully synthesized peptide derivatives containing the thioamide function in the backbone, du Vigneaud,[1] Lawesson,[2] and Ried.[3] The synthesis of various protected derivatives of thioasparagine ($H_2NCH(CH_2CSNH_2)CO_2H$) was first reported by Ressler and Banerjee.[4] However, from our own work in this area, it appeared unlikely that thioasparagine-containing peptides could be directly prepared by incorporating activated thioasparagine into growing peptide chains by traditional methods due to the tendency of the thioamide function to enter into intramolecular reactions with activated carboxyl derivatives.[5] Nevertheless, the availability of thioasparaginyl peptides would be of great importance as novel structure-function probes, especially in the case of such peptides as oxytocin, where the asparaginyl side chain has been implicated as an "active element" in the biological function of that hormone.[6]

It appeared that the synthesis of Tan-containing peptides could best be approached by an indirect method. While there are many known methods for the selective conversion of amides to thioamides (in the presence of esters or protecting groups) using such agents as P_2S_5[7] and 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide,[8] these routes are not appropriate in cases where more than one amide function is present. A more reasonable approach for the side chain incorporation of thioamides in peptides seemed to be by using an intermediate cyano function as a thioamide synthon. The approach outlined below utilizes the base-catalyzed hydrogen sulfide addition to nitriles[9,10] under moderate pressure to yield the desired thioasparagine derivatives.

The dipeptide, Boc-L-thioasparaginylglycinamide, was prepared by first synthesizing Boc-L-β-cyanoalanylglycinamide, followed by thioamidation using H_2S/NH_3. The Boc-L-β-cyanoalanylglycinamide (I) was synthesized by condensing glycinamide with Boc-L-β-cyanoalanine-3,4,5 trichlorophenyl



I II

[†]In this work L-thioasparagine and L-β-cyanoalanine are abbreviated Tan and Can, respectively.

ester. The ester was in turn prepared by 1) dehydration of Boc-L-asparagine with dicyclohexylcarbodiimide (DCC) in pyridine and dimethyl formamide and 2) its subsequent activation with trichlorophenol using DCC in dimethylformamide and ethyl acetate at room temperature for 36 hours under anhydrous conditions, without isolation of the intermediate acid. This dipeptide (I) (0.8g) was dissolved in 30 ml of dry methanol in a thick-walled bottle flushed with dry nitrogen. The mixture was cooled to -70°C, and NH_3 and H_2S gases were bubbled into the solution for 10 min, or until saturated (excess gases result in salt precipitation). The bottle was sealed and the solution was warmed to room temperature and stirred for 48 hours. The excess gases were removed by aspiration and the dipeptide was extracted into 50 ml of EtOAc and the organic phase washed three times each with 25 ml ice water and 25 ml saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to 2 ml. The product was crystallized from ether/hexane to give 0.34 g white solid, II (44%). This method was used in a similar procedure to synthesize Boc-Tan-Phe-NH_2 and larger peptides including Boc-Cys(PMB)-Tan-Gly-NH_2, and Boc-Tan-Cys(PMB)-Pro-Leu-Gly-NH_2 (Table 1), the latter representing a protected C-terminal pentapeptide analogue fragment of oxytocin.

TABLE 1. THIOAMIDE-CONTAINING AMINO ACIDS AND PEPTIDES

STRUCTURE	PREPARATION METHOD	% YIELD	MP °C	$[\alpha]_D^{25}$	$\overset{S}{\overset{\|}{C}}$ ^{13}C c
Thioacetamide[a]	--	--	113-114	--	205.6
Cbz-Tan[4]	H_2S/NH_3	--	123-124	-42.9	--
Cbz-Tan	P_2S_5	--	124-125	-39.1	205.16
Cbz-Tan-Bzl	P_2S_5	48%	107-108.5	-19.6	204.45
Boc-Tan-Gly-NH_2	H_2S/NH_3	44%	173-174.5	+20.0	205.7
Boc-Tan-Phe-NH_2[b]	H_2S/NH_3	45%	185-187	-37.8	204.85
Boc-Tan-Cys(PMB)-Pro-Leu-Gly-NH_2	H_2S/NH_3	60%	123-124	-73.0	204.88
Boc-Cys(PMB)-Tan-Gly-NH_2	H_2S/NH_3	70%	74-77	--	205.83

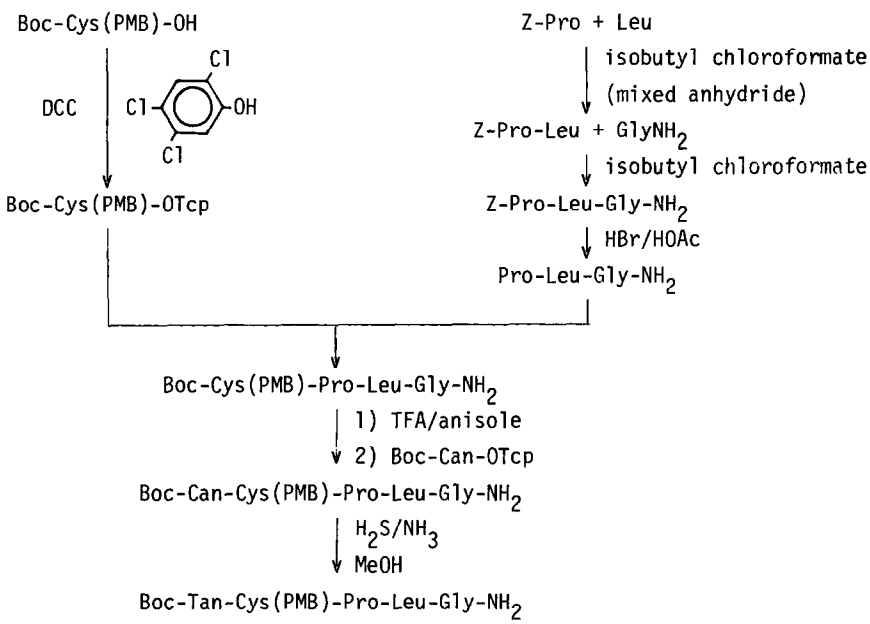
All new compounds were characterized by elemental analyses (C, H, N, S) and/or mass spectroscopy; homogeniety was ascertained by proton and carbon nmr spectroscopy and by tlc in at least two dfiferent solvent systems. Some decomposition was noted on silica gel G plates. a) This result is from lit. b) Isolated from H_2S/NH_3 treatment of Boc-Can-PheOCH_3. c) Downfield in ppm from tetramethysilane.

The conversion of the nitrile functional group to the thioamide can be confirmed by C-13 NMR by observing the disappearance of the nitrile carbon (~117 ppm-TMS) and the appearance of the thioamide carbon (~205 ppm-TMS, Table 1).

In each case, the N-α-Boc-protected β-cyanoalanine moiety was introduced within the growing peptide chain by the active ester methodology, using its 2,4,5-trichlorophenyl ester. In the

synthesis of the thioasparaginyl pentapeptide, the initial tripeptide Pro-Leu-GlyNH_2 was first prepared by the method of Cash.[11] Next the tetrapeptide Boc-Cys(PMB)-Pro-Leu-Gly-NH_2 was synthesized by condensation of the activated and protected cysteine derivative as shown in Scheme I. The further elongation of the peptide with β-cyanoalanine (Can) gave the protected pentapeptide which was converted to the thioasparagine (Tan)-containing derivative in similar fashion to that described above in 70% yield.

SCHEME I. SOLUTION SYNTHESIS OF A TAN-CONTAINING PENTAPEPTIDE



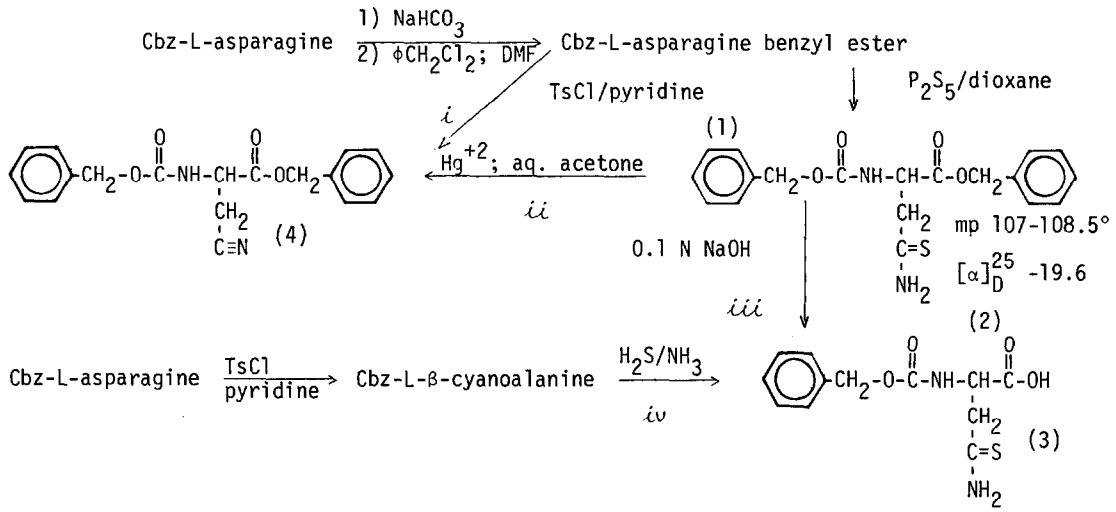
Attempts to remove the N-α-tert-butyloxycarbonyl group from N-terminal Tan derivatives using the standard trifluoroacetic acid method resulted in some decomposition of the thioamide group as monitored by C-13 NMR (disappearance of the thioamide carbonyl). A preferred approach would involve synthesis of a larger peptide containing β-cyanoalanine at an interior position with conversion to the thioamide as one of the final steps in the synthesis. This approach was successfully utilized in the synthesis of the tripeptide Boc-Cys(PMB)-Tan-GlyNH_2 (Table 1).

Since this reaction was carried out in basic conditions, racemization is possible. In order to establish the stereochemical integrity of the products, several key intermediates were compared following their synthesis by alternate routes. Thus, as shown in Scheme II, the Cbz-thioasparaginyl benzyl ester (2) was synthesized by the P_2S_5 method and reconverted to its β-cyanoalanyl derivative (4) by treatment with mercuric acetate in acetone. This comparison provided evidence that the P_2S_5 procedure does not affect chirality of the α-carbon. Next the Cbz-thioasparaginyl benzyl ester derived from the P_2S_5 route (2) was saponified with 0.1 N sodium hydroxide to yield Cbz-thioasparagine. This product was similar in both physical and optical properties to a sample prepared by the H_2S/NH_3 thioamidation treatment,[4] thereby confirming the utility of each of these

two procedures.

It may therefore be concluded that the indirect conversion of peptides containing β-cyanoalanyl moieties to their corresponding thioamide derivatives represents a convenient and viable approach to thioasparaginyl peptides.

SCHEME II. SYNTHETIC ROUTES LEADING TO THIOASPARAGINE DERIVATIVES: EVIDENCE FOR PRESERVATION OF STEREOCHEMICAL INTEGRITY.



4 via route	mp	$[\alpha]_D^{25}$
i.	101-103°	-24.4
ii.	101-102.5°	-23.5

3 via route	mp	$[\alpha]_D^{25}$
iii.	124-125°	-39.1
iv.	123-125°	-42.9

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