A NEW SILYLATION REAGENT FOR AMINO ACIDS BIS(TRIMETHYLSILYL)TRIFLUOROACETAMIDE (BSTFA)[1,2]

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It is not generally known that the trimethylsilyl group was introduced by Rühlmann (1961) for the GLC analysis of amino acids before its application in carbohydrate chemistry, Bentley, et al. (1963). Until 1965, only a few researchers used the TMS derivative with limited success due to the instability of the N-TMS group and the non-availability of an effective silylating reagent.

Following the development of some new silylating reagents studies were renewed on the preparation of the TMS derivatives of the amino acids. The TMS derivative holds considerable promise as the reaction is complete in one step. Smith and coworkers (1965,1966) investigated the reaction conditions for the formation of the trimethylsilyl derivatives of amino acids using hexamethyldisilizane (HMDS) and trimethylchlorosilane (TMCS) with different catalysts. They also studied trimethylsilyldiethylamine (TMSDEA) which gave the highest yields, from 89 to 99%, for leucine, serine, and aspartic acid.

In 1966, Klebe, et al. reported studies on the synthesis and application of the now widely used silylating reagent, N, O-bis (trimethylsilyl) acetamide (BSA), and obtained peaks for 19 of the protein amino acids. However, the derivatives of glycine

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[3]Experimental data taken in part from masters thesis, University of Missouri, June 1968.

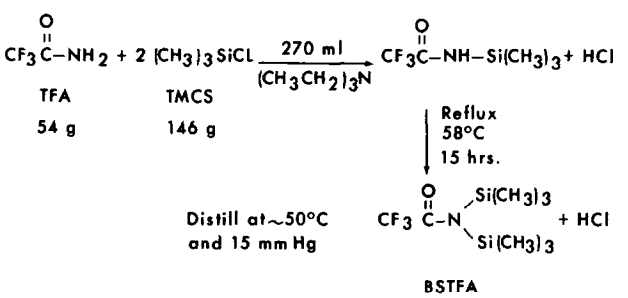
and alanine could not be separated from one of the reaction products of BSA, mono(trimethylsilyl) acetamide (MSA), due to its similar chromatographic retention. In 1967, Birkofer and Donike suggested N-TMS-N-methyl acetamide and N-TMS-N-methyl formamide as silylating reagents.

To overcome the interference due to MSA we have synthesized a new chemical reagent, bis(trimethylsilyl)trifluoroacetamide (BSTFA). This reagent was investigated as to the reaction conditions required for the precise and quantitative formation of the amino acid TMS derivatives.

Synthesis and Chromatography of BSTFA- Figure 1 gives the preparation of BSTFA. The trifluoroacetamide was placed in a three liter, three-necked round bottomed flask with ground glass fittings. An overhead stirrer, a 150 ml addition funnel, and a condenser capped with a $CaSO_4$ drying tube were placed on the flask. The anhydrous triethylamine was placed in the flask, then the TMCS was slowly added through the addition funnel over a period of 30 minutes. After refluxing at ca. 58° C with constant stirring for 15 hours, the reaction mixture was filtered through a Buchner funnel under a N_2 atmosphere. The BSTFA was obtained from the filtrate by vacuum distillation at 45 to 50° C at 14 mm Hg.

FIGURE 1

SYNTHESIS OF BIS(TRIMETHYLSILYL)TRIFLUOROACETAMIDE



A chromatographic comparison of BSTFA and BSA is shown in Figure 2. Since BSTFA and its reaction product MSTFA are considerably more volatile, interference with the chromatographic separation of alanine and glycine does not occur. Also, as the number of moles of amino acids to be derivatized increases, more

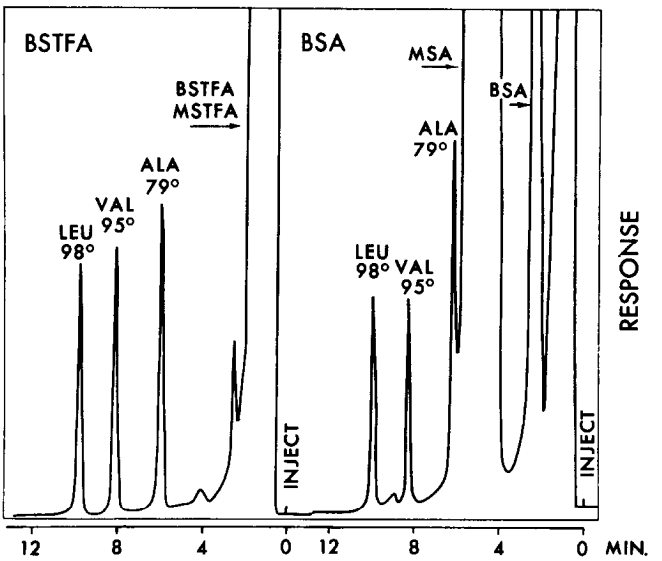
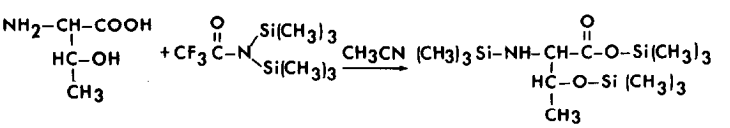


FIG. 2 CHROMATOGRAPHIC INTERFERENCES USING BSA AND BSTFA SEPARATED WITH 3.0% (w/w) DC—550 ON 80/100 MESH HIGH PERFORMANCE CHROMOSORB G. 1m×4mm i.d. GLASS. 60°C INITIAL TEMP., 5°C/min., N_2, 70 ml/min.

BSA is required and the interference from MSA is then more pronounced. Further, BSTFA is completely miscible with acetonitrile, whereas a 3:1 v/v ratio of CH_3CN to BSA is required for complete miscibility. Figure 3 shows the formation of the TMS derivative of threonine.

FIGURE 3
TRIMETHYLSILYLATION OF THREONINE



CLOSED TUBE, 125°C, 15 MIN CH_3CN/BSTFA, 1:1 v/v

Experimental- The molar response for each amino acid relative to phenanthrene was determined at 125 and 150° C at reaction times of 5, 15, and 30 minutes. The amino acids were silylated in

16 x 75 mm, No. 9826 culture tubes with teflon lined caps. The sample tubes were placed in a constant temperature oil bath (±1°C) and immersed just to the liquid level. Caution: Place a safety shield in front of the oil bath.

Silylation Method - General

(a) Place 1.00 mg of dry amino acid or hydrochloride and an exact amount (ca. 1.00 mg) of phenanthrene as an internal standard in the reaction tube.
(b) Add 0.2 ml of CH_3CN and 0.2 ml of BSTFA.
(c) Securely cap the tube and place it in the oil bath just to the liquid level.
(d) Heat at 125° C for 15 minutes, remove the tube and allow to cool.
(e) Wipe the exterior of the tube with a hexane dampened tissue to remove the oil film.

The protein amino acids were silylated with BSTFA singly and in mixtures. No interactions were noted. The samples were chromatographed on a 1.0 m x 4 mm i.d. borosilicate glass column packed with 3.0% w/w DC-550 coated on 80/100 mesh H.P. Chromosorb G with a N_2 carrier flow of 70 ml/min. The TMS derivatives eluted from 90 to 230° C. Elution occurred at higher temperatures on 4.0% w/w SE-30 columns coated on the same support material. The peak areas were determined by integration with an Infotronics Model CRS-11AB/HS/42 digital integrator system.

Results and Discussion- Chromatographic peaks were obtained for all of the protein amino acids except arginine. Table I summarizes the data obtained from the silylation studies at different times and temperatures. Excellent agreement for individual samples was obtained at 150° C for 5, 15, and 30 minutes. Only glycine, glutamic acid, and cystine showed significant variations in response as a function of time and temperature. Two chromatographic peaks were obtained for glycine and glutamic acid when silylated at 150° C. The ratio of the peak area of the later eluting peak increased relative to the first peak with increasing reaction times at 150° C. The second peak for glycine and glutamic acid did not form at 125° C with reaction times of 5 or 15 minutes. Excellent agreement was obtained for the RMR of glycine and glutamic acid at 125° C for 5 or 15 minutes, and for glutamic acid at 70° C for 30 to 90 minutes.

Since BSTFA is a bis substituted amide and a superior silyl group donor, derivatization of glutamine and asparagine was possible. The reproducible synthesis and chromatographic resolu-

TABLE I. RELATIVE MOLAR RESPONSE OF THE TMS AMINO ACID DERIVATIVES

Amino Acid Class	RMR[a,b] at 150°C, min.				125°C, min.
	5	15	30	Av.	15
I. Aliphatic					
Glycine	0.46	0.34	0.28	0.36	0.45
Alanine	0.65	0.64	0.64	0.64	0.62
Leucine	0.80	0.80	0.81	0.80	0.82
Isoleucine	0.73	0.73	0.72	0.73	0.75
Valine	0.72	0.71	0.75	0.73	0.75
Phenylalanine	0.99	1.00	1.02	1.00	0.99
II. Dicarboxylic					
Aspartic Acid	0.85	0.84	0.86	0.85	0.86
Glutamic Acid	0.68	0.57	0.42	0.56	1.00
III. Hydroxy					
Serine	0.87	0.88	0.87	0.87	0.89
Threonine	1.03	1.02	1.00	1.02	1.04
Tyrosine	1.20	1.20	1.22	1.21	1.18
IV. Sulfur					
Cysteine	0.85	0.85	0.85	0.85	0.84
Cystine	0.00	0.19	0.67[c]		0.10
Methionine	0.62	0.62	0.62	0.62	0.65
V. Basic					
Lysine	0.62	0.65	0.64	0.64	0.65
Arginine	0.00	0.00	0.00	0.00	0.00
VI. Heterocyclic					
Proline	0.58	0.57	0.60	0.58	0.62
Tryptophan	0.28	1.31	1.25	1.28	1.26
Hydroxyproline	0.94	0.93	0.90	0.92	0.94
Histidine	0.75	0.75	0.81	0.77	0.76
$AspNH_2$ (150°C)	0.39	0.52	0.51		
$GluNH_2$ (70°C)	0.65(30 min.)		0.64(60 min.)		0.67(90 min.)

Column: 3.0% w/w DC-550 on 80/100 mesh high performance Chromosorb G, 1.0 m x 4 mm i.d. glass.

[a]Each value is an average of 2 or more independent runs. Average sigma of 0.0258. RSD range of 1.49 to 3.09%.

[b] $RMR_{aa/phen} = \frac{Area_{aa/mole}}{Area_{phen/mole}}$

[c]Same value for 120 min.

tion of the TMS derivatives of both of these biologically important compounds has not been previously reported. These two amides were not silylated at the same reaction conditions found suitable for the other amino acids. $AspNH_2$ was best converted to the TMS derivative in 30 minutes at 150° C and $GluNH_2$ in 30 minutes at 70° C (Fig. 4). Multiple peaks were observed for glutamine when

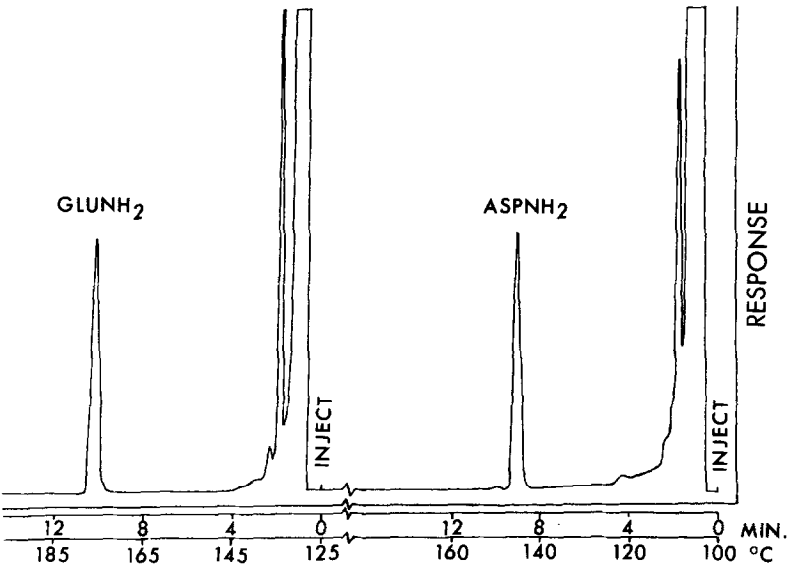


FIG. 4 CHROMATOGRAM OF TMS DERIVATIVES OF ASPARAGINE AND GLUTAMINE COLUMN: 4.0% (w/w) SE-30 ON 80/100 MESH HIGH PERFORMANCE CHROMOSORB G. 1.0m × 4mm i.d., GLASS, N_2 FLOW 75 ml/min.

silylated at temperatures of 100 to 150° C for 5 minutes or longer. The RMR data are presented in Table I.

Summary and Conclusions - A new silylating reagent bis(trimethylsilyl)trifluoroacetamide (BSTFA) has been synthesized and evaluated for the preparation of amino acid TMS derivatives. BSTFA is an active silylating reagent; has increased volatility and appears with the solvent front; has a lower detector response and greater solubility in some solvents than BSA. The fluorine in BSTFA results in less SiO_2 deposits and thus decreased detector noise. Silylation at 125° C for 15 minutes in a closed tube resulted in single reproducible derivatives for 18 of the 20 protein amino acids, and cystine was derivatized in 30 minutes at 150° C. No reproducible chromatographic peak was obtained for arginine. $AspNH_2$ was converted to the TMS derivative in 30 minutes at 150° C, $GluNH_2$ in 30 minutes at 70° C. BSTFA and its reaction product MSTFA are more volatile than BSA and do not interfere in the chromatographic separation of alanine and glycine. Nonpolar liquid phases must be used in chromatography of the TMS-amino acid derivatives as decomposition occurs on polyester columns.

Silylation of amino acids and other biologically important molecules with BSTFA holds considerable promise as the derivatives can be prepared in a single step with little time. Separation studies are now in progress and will be the subject of a later paper.

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