Tetrahedron Vol. 42, No. 2, pp. 547 to 552, 1986
Printed in Great Britain.

0040-4020/86 $3.00 + .00
Pergamon Press Ltd.

A MODIFIED SYNTHESIS OF MOSHER'S ACID AND ITS USE IN A SENSITIVE STEREOISOMER ANALYSIS OF AMINO ACID DERIVATIVES

William E. Hull,
Bruker Analytische Meßtechnik GmbH
7512 Rheinstetten 4-Fo.
Present adress: Institut für Biochemie, DKFZ,
6900 Heidelberg

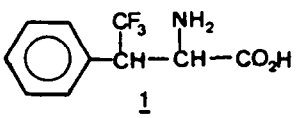
Klaus Seeholzer, Marlies Baumeister and Ivar Ugi,*
Organisch-Chemisches Institut der Technischen
Universität München, D-8046 Garching

(*Received in Germany* 24 *April* 1985)

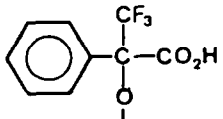
Abstract:
A convenient and sensitive method for stereoisomer analysis of amino acid derivatives is described. It is based on the ^{19}F NMR-analysis of mixtures containing epimers of N-.-methoxy-.-trifluoromethylphenylacetyl alanine (MTPA). An improved procedure for the synthesis of MTPA is given.

Introduction:

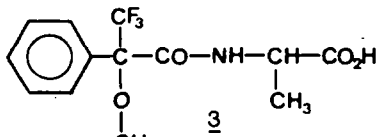
Racemization tests for peptide syntheses by spectroscopic methods are generally much faster and easier to execute than "wet" racemization tests. Due to signal overlap, the analysis of racemized peptide derivatives through ^{1}H NMR[1] is possible only in a few cases, and ^{13}C NMR is here also of limited usefulness[2]. Sievers, Bayer and Hunziker[3] have found that the ^{19}F NMR spectra of diastereomeric N-TFA-peptides differ in ^{19}F- chemical shift. Although the observed differences were too small (.. = 0,02 - 0,03 ppm) for a convenient quantitative ^{19}F NMR racemization test, they indicate the possibility of analyzing mixtures of suitably ^{19}F-labelled peptide derivatives by ^{19}F NMR. This led Stüber[4] at our laboratory to look for a chemical system that may serve as a ^{19}F-probe in ^{19}F-NMR racemization tests. According to preliminary results, the two epimers of **1** differ considerably in their ^{19}F chemical shifts.



1



2



3

One of these epimers is thermodynamically labile and shows a strong tendency to epimerize. The same holds for most known derivatives of **1**. This is a good candi-

date for a 19F-probe of racemization tests. However, it is too difficult to prepare the labile epimer of 1 in sufficient purity[5].

Mosher[6] et. al proposed the use of chiral α-methoxy-α-trifluoromethyl-phenylacetic acid 2 (MTPA, Mosher's acid) as a reagent for the determination of enantiomer purity of chiral amines.

Breuer and Ugi[7] studied the N-MTPA derivatives of various α-amino acids in regard to their suitability as ^{19}F probes; it was found that the epimeric MTPA derivatives of aliphatic amino acids, in particular 3, as well as its amides and peptide derivatives differ sufficiently in ^{19}F chemical shift ($\Delta\delta$ = 0.32 - 0.40 ppm) to serve as the basis of a ^{19}F NMR racemization test. The use of a variety of methods in the synthesis of peptide derivatives from 3-(R,S) and ^{19}F-analysis of the products demonstrated that 2 is, in fact, very well-suited as a reagent for ^{19}F-NMR racemization tests. Such tests are fast and easy to conduct; its usefulness was, however, up to now severely limited, because its sensitivity was no better than 2 - 3 %, when the customary ^{19}F NMR techniques and equipment were being used[5,7].

Results and Discussion

The present paper deals with an improvement in sensitivity[7] of epimer detectability level, through which this method becomes a racemization test with specific advantages. Since the epimers of 3, and related compounds have very similar ^{19}F-NMR properties, it suffices to confine these sensitivity studies to 3 itself as the model compound.

Conclusion

It has been demonstrated that ^{19}F NMR provides a convenient means of assaying diastereomer purity in peptide synthesis by virtue of the distinguishable ^{19}F-chemical shifts for (R,R) and (R,S) N-MTPA derivatives of amino acids. The minor diastereomer can readily be detected at levels as low as 0.05 % with am accuracy of 0.005 % in 0.4 ml samples containing a 0.082 molar concentration of MTPA derivative.

Table. The analysis of epimer mixtures of 3 by 376 MHz ^{19}F-NMR.

Mol. % 3-(R,R) in a mixture of 3-(R,S) and 3-(R,R) (by weight)b)	Composition according to ^{19}F NMR data[a)] (% 3-(R,R))	aquisition-time (min.)
0.00	0.039 - 0.004	11.3
0.125	0.124 - 0.001	7.0
0.500	0.52 - 0.02	2.8
1.00	0.98 - 0.02	2.8

a) The limits of error here are not standard deviations but rather bounds computed from maximum integrals (see experimental section.)

b) The reference isomer 3-(R,S) contained according to ^{19}F-NMR ca. 0.04 % of 3-(R,R).

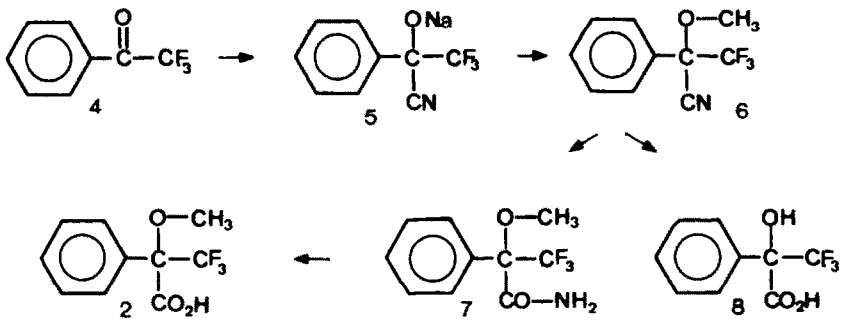
A high-field (9.4 Tesla) NMR spectrometer was used in the development of the present method. However, any superconducting magnet system of the 200 - 360 MHz class will certainly suffice for the ^{19}F NMR measurements. Even electromagnets of the 80 - 100 MHz class will be usable, if a sensitivity of ca. 0.5% and prolonged

recording times are accepted. With the latter equipment ^{1}H-decoupling may be necessary, and the results will strongly depend upon the quality of the magnet.

The data of the above table indicate that the ^{19}F-NMR method meets the sensitivity requirements of peptide synthesis.

The synthesis of MTPA by the original method[6] is based on the sequence 4 ⟶ 5 ⟶ 6 ⟶ 2 of reactions.

For the preparation of the MTPA used here, a synthesis was developed which contains some minor improvements over the original preparation.



Since the synthesis of **4** by the published Friedel-Crafts procedure yields only 41% **4**[8], trifluoroacetophenone **4** is usually prepared in 63 % yield from phenyl magnesium bromide and trifluoroacetic acid[9]. We have developed a Friedel-Crafts procedure for **4** with a yield of 76 %. Thus the Friedel-Crafts method has become a viable alternative to the customary preparation.

As published[6], **4** is converted into **6** in 1.2-dimethoxyethane solution in 97 % yield; with this method we obtained 65 - 70 % on the average. The conversion of **4** into **6** proceeds in tert-butanol, a much less expensive solvent, with a 85 % yield.

We observed that the formation of **2** by acid catalyzed hydrolysis of **6** (63 %) is accompanied by formation of **8** (about 20 %) as a side reaction. It is avoidable through hydrolysis of **6** in alkaline solution; alkaline hydrogen peroxide[10] converts **6** into **7** which is hydrolyzable by aqueous alkali with a yield of 84 %, based on **6**.

Racemic MTPA is resolved with (R)-α-phenyl-ethyl amine[6]. From the (R)-MTPA thus obtained **3-(R,S)** and **3-(R,R)** are prepared in 99,96 % epimer purity. The above table contains ^{19}F NMR data of their mixtures.

Experimental

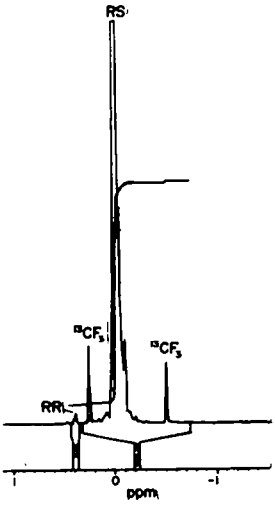
NMR Spectra

The ^{19}F NMR spectra were measured with a Bruker AM-400 FT NMR spectrometer at 376,5 MHz and 297 ^{o} K. The samples of the above table had a concentration of 25 mg/ml in $CDCl_3$ and a volume of 0.4 ml in the 5 mm tubes used.

A standard 5 mm ^{1}H-probe was tuned to the ^{19}F-frequency, using the external tuning

control, and the ^{19}F NMR spectra were measured without ^{1}H-decoupling.

The ^{1}H-^{19}F long range coupling between CF_3 and C_6H_5 groups of MTPA resulted in a linewidth of 5.4 Hz. Customary Fourier transform NMR techniques were used: spectral width 5 KHz for 16 K data points with quadrature detection, 16 bit A/D converter for better dynamic range behaviour, pulse angle ca. 30° (3 usec), aquisition time 1.64 sec, relaxation time 1.0 sec, number of transients per FID 64 - 256.



For improved accuracy on ^{19}F-signal integration, each FID was multiplied by a Lorentz-Gauss-line-shape transformation function[11] which was selected to improve resolution without influencing the ratios of the inte(Platz f. Formel) grals. After Fourier transformation one finds a main signal of the MTPA derivatives 3-(R,S) surrounded by two $^{13}CF_3$ ^{13}C-satellites ($^{J}CF_3$ = 290.1 Hz, isotope shift $\Delta\delta$ = 0.127 ppm). The signal of 3-(R,R) is 0.319 ppm downfield (see spectrum in fig). Furthermore, some contaminations were found at 0.975 ppm, (0.03 %).

Fig. ^{19}F-NMR of 3-(R,S) containing 0.12 %3-(R,R).

The integral of the 3-(R,R) $^{12}CF_3$ signal alone (without correction for its unobserved non-integrated $^{13}CF_3$ satellites) yields directly the data in our table.

Trifluoroacetophenone 4

At 20° C 114 g (1.0 mol) trifluoroacetic acid are added in ca. three h to a well-stirred suspension of 26 g (125 mol) phosphorus pentachloride in 400 ml 1.2-dichloroethane. The trifluoroacetylchloride thus formed (bp. - 2 ° C) is condensed in a dry ice/methanol cooled trap (215 g, 81 %), from which it is subsequently destilled into a reaction vessel within three hours. The vessel is completely immersed in a cooling bath to maintain -40° C ± 3°C within the vessel and it is fitted with a dry ice/methanol cooled reflux condenser. If the reaction mixture reaches > -30° C, even locally, it tends to turn black, and a low yield is obtained. The vessel contains a suspension of 135 g (1.00 mol) finely ground aluminium chloride. The aluminium chloride is ground in suspension with an Ultra-Turrax high-speed mixer (Janke & Kunkel, Stauffen, W.-Ger.) in 300 ml benzene and 120 ml carbon disulfide. The reaction mixture is stirred with a Hershberg stirrer[12]. After another three hours at -40° C, the reaction mixture is poured into a stirred mixture of 300 ml conc. hydrochloric acid and 300 g of ice. The phases are separated and the aqueous solution is extracted with ether and benzene. The combined organic extracts are washed with saturated aq. sodium hydrogen carbonate and then dried over magnesium sulfate. Distillation yields 107 g (76 %) trifluoroacetophenone.
Bp. 46 - 49° C/15 torr.

α-Methoxy-α-trifluoromethyl-phenyl-acetonitrile (MTPA nitrile) 6

The mixture of 50 g (0.29 mol) trifluoroacetophenone, 20 g (0.41 mol) sodium cyanide and 200 ml tert.-butanol is converted into a fine suspension with an Ultra-Turrax mixer. Over a period of 2 h 40 ml (0.42 mol) freshly distilled dimethyl sulfate are added to the stirred suspension; the temperature is not allowed to rise above 60° C. After addition of 100 ml pentane the solids are removed by filtration. Distillation in vacuo yields 52.3 g (85 %) of α-methoxy-α-trifluoro-methyl-phenylacetonitrile (b.p.80 - 82o C/15 torr).

α-Methoxy-α-trifluoromethyl-phenyl-acetic acid (MTPA)2

At 0° C 30 ml 35 % aqueous hydrogen peroxide are added to a stirred solution of 50 g (0.125 mol) sodium hydroxide in 120 ml ethanol and 10 ml water. After one hour at 0° C the reaction mixture is refluxed for two h. After evaporation of the solvent the residue is refluxed for five h with 40 g (1.00 mol) sodium hydroxide in 120 ml water. The reaction mixture is diluted with 120 ml water and extracted with 100 ml ether. After acidification to pH = 3 with 20 % aqueous sulfuric acid, the MTPA is extracted by three 100 ml portions of diethyl ether/dichloromethane (3 + 1, v., v.). After drying ($MgSO_4$) the solvent is evaporated and the residue is distilled in vacuo (b.p. 83 - 85° C/ 0.5 Torr, bath in 140° C). Yield = 45,5g (84 %).

REFERENCES

1. B. Halpern, L.F. Chew and B. Weinstein, J. Amer. Chem. Soc., **89**, 5051 (1967); B. Weinstein and A.E. Pritchard, J. Chem. Soc., Perkin Trans. I **1972**, 1015; N.L. Benoiton, K. Kuroda and F.M. Chen, Int. J. Peptide Protein Res., **13**, 403 (1979); N.L. Benoiton, Kuroda, ibid., **17**, 197 (1981); R. Nagaraj and P. Balarm, Tetrahedron, **37**, 2001 (1981).

2. J.S. Davies, R.J. Thomas and M.K. Williams, Chem. Comm., **1975**, 76; R. Deslaurierts, J.C.P. Smith, R.L. Somorjai, E. Raboton, R.C. Orlowski and R. Water, Int. J. Peptide Protein Res., **13**, 473 (1979).

3. R.E. Sievers, E.Bayer and P. Hunziker, Nature, **223**, 179 (1969); see also: E. Bayer, P. Hunziker, M. Mutter, R.E. Sievers and R. Uhmann, J. Amer. Chem. Soc., **94**, 265 (1972).

4. S. Stüber, Dissertation, Techn. Universität München, 1976.

5. W. Breuer, Dissertation, Techn. Universität München, 1981.

6. G.R. Sullivan, J.A. Dale and H.S. Mosher, J. Org. Chem., **38**, 2143 (1973); J.A. Dale, D.L. Dull and H.S. Mosher, J. Org. Chem., **34**, 2543 (1969).

7. W. Breuer and I. Ugi, J. Chem. Research (s) **1982**, 271; (M), **1982**, 2901.

8. J.H. Simons, W.T. Black and R.C. Clark, J. Amer. Chem. Soc., **75**, 5621 (1953).

9. K.T. Dishart and R. Levine, ibid., 78, 2268 (1956).

10. in analogy to: P.L. Compagnon and M. Miocque, Ann. Chim., 5, 11 (1970); C.R. Noller, Org. Synth., 2, 586 (1943).

11. R.R. Ernst, Advances in Magnetic Resonance, ed.: J.S. Wangh, Vol 2, p.1, Academic Press, New York 1966.

12. E.B. Hershberg, Ind. Eng. Chem., Anal. Ed., 8 313 (1936); Org. Synth., Coll. Vol. 2 115 (1943).

Acknowledgement:
We acknowledge gratefully the financial support for our work by Deutsche Forschungsgemeinschaft, SFB 145 and the Fonds der Chemischen Industrie.