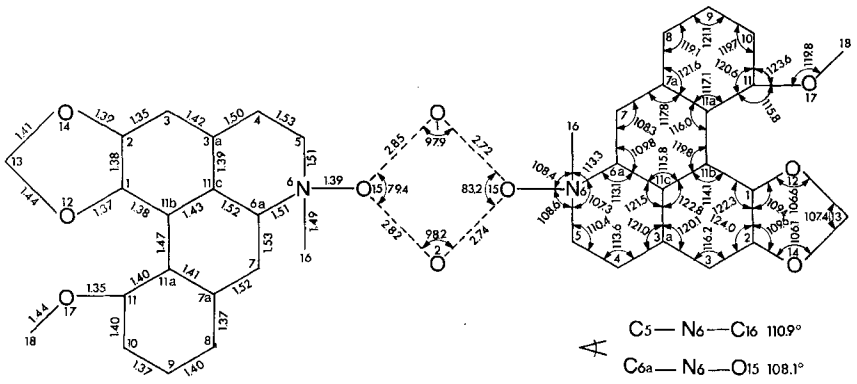
Methoxy-Gruppe liegt ungefähr in der Ring-D-Ebene. Die Ebenen des Biphenylsystems sind um einen Winkel von 32,3° bzw. 34,4° gegeneinander verdreht. Ganz ähnliche Verhältnisse liegen auch im Bulbocapnin-methojodid [7] [9] vor.

Die über die beiden unabhängigen Molekeln in der asymmetrischen Einheit der Zelle gemittelten Bindungslängen und Bindungswinkel finden sich in Figur 3.




*Bindungslängen* (e.s.d. ≅ 0,01 Å) *Bindungswinkel* e.s.d. ≅ 0,7°)

Fig. 3

LITERATURVERZEICHNIS

[1] *Ek. Weiss, Karl Bernauer & A. Girardet*, Helv. *54*, 1342 (1971).
[2] *M. Shamma & M. J. Hillmann*, Experientia *25*, 544 (1969).
[3] *A. J. C. Wilson*, Nature *150*, 152 (1942).
[4] *I. L. Karle, K. S. Dragonette & S. A. Brenner*, Acta crystallogr. *19*, 713 (1965).
[5] *J. Karle & I. L. Karle*, Acta crystallogr. *21*, 849 (1966).
[6] *G. Germain & M. M. Woolfson*, Acta crystallogr. *B 24*, 91 (1968).
[7] *M. Shamma & W. A. Slusarchyk*, Chem. Reviews *64*/1, 72 (1964).
[8] *W. Klyne & V. Prelog*, Experientia *16*, 521 (1960).
[9] *T. Ashida, R. Pepinsky & Y. Okaya*, Acta crystallogr. *16*, A 48 (1963).

## 144. *p*-Azido-L-phenylalanine: a Photo-affinity 'Probe' Related to Tyrosine

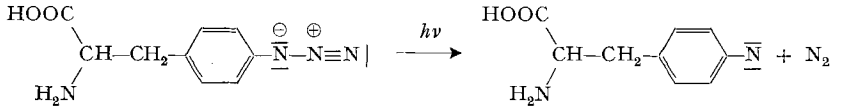
by **Robert Schwyzer** and **Mario Caviezel**

Institut für Molekularbiologie und Biophysik
Eidgenössische Technische Hochschule, 8049 Zürich

(23. III. 71)

*Zusammenfassung.* Synthese und Eigenschaften des *p*-Azido-L-phenylalanins (**1**) und einiger seiner für die Peptidsynthese geeigneter Derivate werden beschrieben. Infolge der photolytischen Spaltbarkeit in Stickstoff und das Imen **2** dürfte dieses Analogon des Tyrosins als «photochemische Affinitätssonde» verwendbar sein.

*p*-Azido-L-phenylalanine **1** (Phe($N_3$)[1]), is an L-tyrosine analogue containing an azido in place of a hydroxyl group. The azido group is chemically quite inert, but its photolysis yields the highly reactive nitrene or imene **2**[2]):

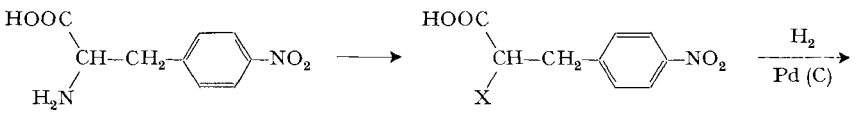


**1** **2**

In this paper, the synthesis of this new amino acid is described and also some of its derivatives, which could serve as intermediates in the preparation of polypeptides.

We envisage the introduction, in biologically active peptides, of *p*-azido-L-phenylalanine in place of aromatic amino acids, especially tyrosine, thus rendering the peptides highly photoactive and so useful for detecting substrate-enzyme, hormone-receptor, antigen-antibody, and other interactions. This is concurrent with our work on hormone derivatives and analogues which make evident interactions with potential receptors and metabolising enzymes, *e.g.* diazoacetyl choline bromide [1] and $N^{\varepsilon}$-dansyllysine[21]-corticotropin-(1-24)-tetrakosipeptide [2]. *Knowles* [3] has introduced $N^{\varepsilon}$-(2-nitro-3-azido-phenyl)-L-lysine as photo-affinity marker for antigen-antibody interactions and has pointed out the special merits of substituted phenyl azides for this purpose. Singer [4] has used similar groups in connection with the study of the acetyl cholinesterase of erythrocytes.

*p*-Nitro-L-phenylalanine **3** was prepared according to *Bergel* [5] and its α-amino group was substituted to give the *t*-butoxycarbonyl- and phthaloyl-derivatives **4** and **4a**. These were directly converted to the dicyclohexylamine (DCHA) salts of the corresponding derivatives **5** and **5a** of *p*-azido-L-phenylalanine **1**, by catalytic hydrogenation of the nitro to the aromatic amino group and subsequent diazotisation and replacement of the diazonium group by means of hydrazoic acid. *t*-Butoxycarbonyl-*p*-azido-L-phenylalanine (Boc · Phe($N_3$) · OH; **5**), and phthaloyl-*p*-azido-L-phenylalanine (Pht > Phe($N_3$) · OH; **5a**), were readily obtained by acidification and extraction of aqueous solutions of the DCHA salts. **5** was converted to its active *p*-nitrophenyl (**6**) and N-hydroxysuccinimide (**6a**) esters using the dicyclohexylcarbodiimide method [6]:

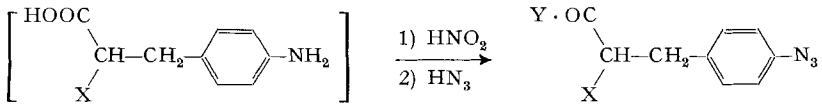


**3** **4** X = $(CH_3)_3COCO \cdot NH-$

**4a** X =

[1]) Abbreviations according to the recommendations of the IUB-IUPAC Joint Commission, cf. European J. Biochemistry *1*, 375 (1967).

[2]) Results of the photolysis of **1** and of peptides containing **1** will be published in forthcoming papers.



**5** X as in **4**, Y = HO–

**5a** X as in **4a**, Y = HO–

**6** X as in **4**, Y = $NO_2$–C$_6$H$_4$–O–

**6a** X as in **4**, Y = $CH_2CO$>N–O– ($CH_2CO$)

Some of the properties of these compounds are listed in the table.

With regard to synthesis it seems rather remarkable that (the intermediary) Boc · Phe($NH_2$) · OH can be subjected to the acidic conditions necessary for conversion to a diazonium salt without decomposition of the *t*-butoxycarbonyl moiety. This is due to the conditions used: low temperature and sufficiently dilute aqueous HCl.

The *p*-azidophenyl group is easily recognised in the IR. and NMR. spectra of the corresponding compounds. The dicyclohexylamine salts of Boc · Phe($N_3$) · OH (**5**) and of Pht > Phe($N_3$) · OH (**5a**), as well as the N-hydroxysuccinimide ester Boc · Phe($N_3$) · OSu (**6**), show typical absorption at 2100 $cm^{-1}$ resulting from the stretching mode of $-N_3$. In the NMR., these compounds exhibit the $AA'BB'$ signals of *p*-substituted benzene with a separation of the mean $\delta A$ from the mean $\delta B$ of approximately 0.33 ppm, whereas this separation is 0.67 ppm in the case of the *p*-nitro substituent (see **4**, exptl.), reflecting the electronegativity difference of the azido and the nitro groups. Typically for such compounds [7], the $AA'BB'$ system is shifted by about 6 Hz towards higher fields as a result of the diamagnetic shielding by the phthaloyl group in Pht > Phe($N_3$) · OH (**5a**) as compared to Boc · Phe($N_3$) · OH (**5**) (as dicyclohexylamine salts).

**Experimental.** – Physical data were determined as follows: Mp. (uncorrected) with an automatic apparatus FP1 of *Mettler* Co., using a very slow rate of heating; optical rotation: *Perkin-Elmer* Polarimeter 141; UV. spectra: *Beckman* Acta V spectrophotometer, solvent ethanol; IR. spectra: *Beckman* IR. 8, solvent chloroform; NMR. spectra: *Varian* T60, solvent deuteromethanol, reference int. tetramethylsilane.

Purity of all compounds was checked by thin-layer chromatography on silica gel using at least two solvent systems. The usual procedure for isolating products from reaction mixtures comprised partition between an aqueous and an appropriate organic phase (generally ethyl acetate), washing at 0–5°C with water and/or acid and/or base according to the properties of the desired product, drying and evaporation of the organic phase in a rotatory evaporator under vacuum. Crystallisation from the solvents listed in the table.

*Boc · Phe($NO_2$) · OH* (**4**). 4.2 g (20 mmoles) of *p*-nitro-L-phenylalanine [5] and 3.16 g (22 mmoles) of distilled *t*-butoxycarbonyl azide were dissolved in a mixture of 15 ml of dioxane and 15 ml of water and kept at pH 10.1 with a pH-stat, see *Schnabel* [8]. Yield 4.3 g of **4** (69%). NMR. ($AA'BB'$): 448.0, 456.6, 488.8, 497.6 Hz; $\delta A$ = 8.21, $\delta B$ = 7.54 ppm. IR.: 1339 (*s*), 1485 (*m*), 1598 (*m*), 1695 (*s*) $cm^{-1}$.

*Pht > Phe($NO_2$) · OH* (**4a**). 2.1 g (10 mmoles) of *p*-nitro-L-phenylalanine were suspended with stirring in 30 ml of dimethylformamide in an atmosphere of $N_2$ and treated slowly with 1.2 g (12 mmoles) of triethylamine, thus creating a very thick suspension of the salt; stirring was continued for another half hour. After cooling the mixture to 0–5°C and treatment with 2.6 g (12

*Some properties of compounds* **1**–**6a**

| No. | Compound | Composition | Analysis | | | | Cristallisation | | M.p. ° | $[\alpha]_{578}^{25°}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mol. Wt. | | C % | H % | N % | from | Aspect | | MeOH |
| **1** | $N_3$ \| H·Phe·OH | $C_9H_{10}N_4O_2$ | Calc. | 52.42 | 4.89 | 27.17 | Water | needles | dec. above | |
| | | 206.206 | Found | 52.51 | 4.88 | 27.03 | | | ~150° | |
| **4** | $NO_2$ \| Boc·Phe·OH | $C_{14}H_{18}N_2O_6$ | Calc. | 54.19 | 5.85 | 9.03 | Diisopropyl ether/ | needles | 107.1 | +7.94° |
| | | 310.30 | Found | 54.53 | 5.83 | 9.25 | hexane | | | $c = 1.55$ |
| **4a** | $NO_2$ \| Pht > Phe·OH | $C_{17}H_{12}N_2O_6$ | Calc. | 60.00 | 3.55 | 8.23 | Methanol/water | platelets | 204.7 | −232.5° |
| | | 340.30 | Found | 59.95 | 3.83 | 8.23 | | | | $c = 1.55$ |
| **5** | $N_3$ \| Boc·Phe·OH | $C_{14}H_{18}N_4O_4$ | Calc. | 54.89 | 5.92 | 18.29 | Cyclohexane | thin needles | 85.4 | +21.5° |
| | | 306.324 | | 55.14 | 6.93 | 18.15 | | | | $c = 2.75$ |
| **5** | $N_3$ \| Boc·Phe·OH, DCHA | $C_{26}H_{41}N_5O_4$ | Calc. | 64.04 | 8.47 | 14.36 | Diethyl ether/ | very thin | 151.5 | +40.8° |
| | | 487.648 | Found. | 64.34 | 8.46 | 14.24 | hexane | needles | | $c = 1.00$ |
| **5a** | $N_3$ \| Pht > Phe·OH, DCHA | $C_{29}H_{35}N_5O_4$ | Calc. | 67.29 | 6.81 | 13.53 | Ethyl acetate/ | prisms | 153.8 | −150.5° |
| | | 517.634 | Found | 67.41 | 6.81 | 13.32 | diethyl ether | | | $c = 1.03$ |
| **6** | $N_3$ \| Boc·Phe·$OC_6H_4$–$NO_2$ (*p*) | $C_{20}H_{21}N_5O_6$ | Calc. | 56.20 | 4.95 | 16.38 | Ethyl acetate/ | thin needles | 141.4 | +0.923° |
| | | 427.419 | Found | 56.41 | 4.92 | 16.23 | isopropanol | | | $c = 1.17$ |
| **6a** | $N_3$ \| Boc·Phe·OSu[a]) | $C_{18}H_{21}N_5O_6$ | Calc. | 53.59 | 5.25 | 17.36 | Benzene/ | undefined | 140.1 | −26.6° |
| | | 403.396 | Found | 53.91 | 5.32 | 17.24 | diisopropyl ether/ hexane | | | $c = 1.05$ |

[a]) HOSu = N-hydroxysuccinimide.

mmoles) of N-ethoxycarbonyl-phthalimide [9], the ice bath was soon removed; total reaction time 12 hours. The solvent was evaporated at 0.01 Torr, and the oily residue treated with warm 0.1 N HCl; cooling produced crystalline **4a**, after recrystallisation: 2.8 g (82%).

*Boc·Phe($N_3$)·OH* (**5**). 6.2 g (20 mmoles) of Boc·Phe($NO_2$)·OH (**4**) were dissolved in a mixture of 150 ml of methanol and 1.5 ml of glacial acetic acid was hydrogenated at 0–5°C with 340 mg of 10% Pd-charcoal. After 3 hours, the educt had been totally hydrogenated, as indicated by thin-layer chromatography on silica gel using $CHCl_3$:$CH_3OH$:$CH_3COOH$ (95:5:3, v:v) as solvent. The product, Boc·Phe($NH_2$)·OH (approx. 20 mmoles), after filtration from catalyst and evaporation of solvent, was dissolved in a mixture of 10 ml of 2 N HCl and 2 ml of water at 0–2°. The diazonium salt was formed by treating with an ice-cold solution of 1.45 g (21 mequiv.) of sodium nitrite in 5 ml of water; total reaction time 30 min. Filtration removed solid by-products, degassing *in vacuo* expelled excess nitrous acid, care being taken to keep the solutions at 0–2° throughout. A solution of 1.30 g (20 mequiv.) of sodium azide in 5 ml of water was then slowly added with stirring, the mixture kept at approximately 0° for 15 min. and the product extracted with ethyl acetate and isolated by the usual procedure. A solution in ethyl ether: petroleum ether (1:1, v:v) was treated with 3.6 g (20 mmoles) of dicyclohexylamine, for conversion to the salt, which after crystallisation at 0° was obtained in 88.7% yield (8.6 g). NMR. (*AA'BB'*): 413.4, 422.0, 433.8, 442.6 Hz; $\delta A$ = 7.29, $\delta B$ = 6.98 ppm. IR.: 1575 (*m*), 1620 (*s*), 1685 (*s*), 2100 (*s*) $cm^{-1}$.

The free acid **5** was obtained as usual using 0.1 N $H_2SO_4$ and ethyl acetate as solvent; yield practically quantitative.

*Pht > Phe($N_3$)·OH* (**5a**). Procedure as for Boc·Phe($N_3$)·OH (**5**) except that the dried concentrated ethyl acetate phase, obtained by the usual procedure, was only concentrated: treatment with dicyclohexylamine at this stage gave the crystalline salt of **5a**. In a typical experiment, 3.1 g (9.4 mmoles) of Pht > Phe($NO_2$)·OH (**4a**) yielded 3.7 g (76%) **5a**. NMR. (*AA'BB'*): 406.2, 414.6, 428.0, 436.8 Hz; $\delta A$ = 7.19, $\delta B$ = 6.85 ppm. IR.: 1500 (*w*), 1622 (*s*), 1697 (*s*), 1765 (*w*), 2100 (*s*) $cm^{-1}$.

*H·Phe($N_3$)·OH* (**1**). Boc·Phe($N_3$)·OH was treated with an excess of approx. 1.5 N HCl in dry ethyl acetate. After half an hour at 0–5°, most of the crystalline hydrochloride of **1** had precipitated; it was separated by filtration, washed with ethyl acetate and diethyl ether, and dried. The product was dissolved in water and treated with a slight excess of pyridine, on standing at ca. 0–5°, crystals of **1** separated out, which were collected, washed with a small amount of water and dried. UV.: $\lambda_{max}$ = 252 nm ($\varepsilon$ = 16000), solvent water.

*Boc·Phe($N_3$)·ONp* (**6**). The acid **5**, obtained from 2.44 g (5 mmoles) of its dicyclohexylamine salt, was dissolved, together with 765 mg (5.5 mmoles) of *p*-nitrophenol, in 15 ml of dry ethyl acetate. To this solution cooled in ice, an ice-cold solution of 1.13 g (5.5 mmoles) of dicyclohexyl carbodiimide in 5 ml of ethyl acetate was added. The mixture was kept at 0° for 2 hours and then for about 15 hours at room temperature. After filtration from dicyclohexyl urea, the solution was subjected to the usual treatment, phenol removed by washing with half-concentrated, ice-cold solution of sodium hydrogen carbonate; yield 1.7 g (80%) of **6**.

*Boc·Phe($N_3$)·OSu* (**6a**). The procedure was that used for the preparation of the nitrophenyl ester **6**. 2.44 g (5 mmoles) of the dicyclohexylamine salt of **5**, 547 mg (5 mmoles) of N-hydroxy succinimide, and 1.3 g (5.5 mmoles) of dicyclohexyl carbodiimide in 20 ml of ethyl acetate produced 1.65 g (82%) of **6a**. Thin-layer chromatogrammes on alumina in $CHCl_3$:$CH_3COCH_3$ (7:3, *v:v*) indicated a pure product, whereas chromatography on silica gel with the same solvent mixture or with $CHCl_3$:$CH_3OH$:$CH_3COOH$ (95:5:3, *v:v*) always resulted in gradual hydrolysis of the active ester to N-hydroxysuccinimide plus **5**. IR.: 1480 (*m*), 1700 (*s*), 1730 (*s*), 1780 (*m*), 2100 (*s*) $cm^{-1}$.

We thank the *Swiss National Foundation* and *CIBA-GEIGY Ltd.*, Basle, for their financial support. Elemental analyses were kindly performed in the laboratories of Dr. *W. Padowetz* and Dr. *W. Hürzeler* (*CIBA-GEIGY Ltd.*). Thanks are also due to our apprentice, Miss *Erika Uster*, for her skillful technical assistance, as well as to Dr. *Regula Keller*, Mrs. *H. Marzoll*, and Mr. *W. Kindlimann* for determination of some of the physical data.

## BIBLIOGRAPHY

[1] *J. Frank & R. Schwyzer*, Experientia *26*, 1207 (1970).
[2] *R. Schwyzer & P. W. Schiller*, Helv. *54*, 897 (1971).
[3] *G. W. Fleet, R. R. Porter & J. R. Knowles*, Nature *224*, 511 (1969).

[4] *H. R. Kiefer, J. Lindstrom, E. S. Lennox & S. J. Singer,* Proc. nat. Acad. Sci. U.S. *67*, 1688 (1970).
[5] *F. Bergel & J. A. Stock,* J. chem. Soc. *1959*, 90.
[6] *D. F. Elliott & D. W. Russell,* Biochem. J. *66*, 49P (1957); *M. Rothe & F. Kunitz,* Liebigs Ann. Chem. *609*, 88 (1957); *M. Bodanszky & V. du Vigneaud,* J. Amer. chem. Soc. *81*, 5688 (1959).
[7] *R. Schwyzer & U. Ludescher,* Biochemistry *7*, 2514, 2519 (1968).
[8] *E. Schnabel,* Liebigs Ann. Chem. *702*, 188 (1967).
[9] *G. H. L. Nefkens, G. I. Tesser & R. J. F. Nivard,* Rec. Trav. chim. Pays-Bas *79*, 688 (1960).

# 145. Etude de composés d'addition d'acides de *Lewis*, XXXIV [1] Composés d'addition de benzoquinone-1,4 avec $TiCl_4$

par **R. Giallonardo** et **Bernard-P. Susz**
Institut de Chimie Physique de l'Université de Genève

(14 V 71)

*Summary.* The adduct 1,4-benzoquinone·$TiCl_4$ has been prepared in $CH_2Cl_2$ solution at about $-60°$. Its IR. spectrum has been recorded at the same temperature. The experimental study of the vibrational frequencies has been completed by the calculation of the fundamental vibrations in the molecular plane, using *Wilson's* FG method, with slightly simplified models of 1,4-benzoquinone·$TiCl_4$ (13 masses) and 1,4-benzoquinone·$2TiCl_4$ (14 masses); analysis by use of internal and symmetry coordinates. An assignment of most of the observed bands is proposed and the conclusion is reached that the complex, when solid, is $(1,4\text{-benzoquinone}\cdot TiCl_4)_n$.

The force constants $F(C{=}O)$ are $9{,}85 \cdot 10^5$ dyne/cm for the quinone and $8{,}8 \cdot 10^5$ dyne/cm for the disturbed carbonyl bond of the polymerized complex in the model proposed.

At ordinary temperature in benzene solution of the components the adduct 1,4-benzoquinone·$TiCl_4$·benzene precipitated; with the help of the models, the fundamental vibrations of its IR. spectrum have been assigned.

**1. Introduction.** – Les recherches sur les composés d'addition entreprises dans notre laboratoire ont porté jusqu'à présent sur une série d'acides de *Lewis* et sur des donneurs électroniques possédant un groupe carbonyle: cétones, esters, acides et chlorures d'acides, aldéhydes [1] à [5].

Il nous a paru intéressant d'étudier le comportement du groupe quinonique avec le tétrachlorure de titane. $TiCl_4$ a été choisi à cause de l'effet assez marqué qu'il était susceptible de provoquer sur la répartition électronique de la liaison carbonyle et sur le nombre d'onde $\omega(C{=}O)$ associé à la présence du groupe carbonyle. La benzoquinone-1,4, qui a fait l'objet de plusieurs études spectroscopiques infrarouges et *Raman*, a été utilisé comme base de *Lewis*.

La littérature, à notre connaissance, est très pauvre en ce qui concerne les composés d'addition de la benzoquinone-1,4 avec des acides de *Lewis*. *Klages* et collaborateurs [6] ont préparé (solvant: $CH_2Cl_2$) les composés benzoquinone-1,4 · $2SbCl_5$ et benzoquinone-1,4 · $SbCl_5$. Ce sont des solides rouges qui, à l'abri de l'humidité et au dessous de $-50°$, sont stables un certain temps mais qui se décomposent rapidement en quelques minutes à la température ordinaire avec dégagement de HCl et noircissement du résidu. D'après *Soumarokova* et collaborateurs [7] la benzoquinone forme en solution benzénique avec $TiCl_4$ à température ordinaire un précipité rouge foncé qui aurait la composition benzoquinone-1,4 · $TiCl_4$. Ces mêmes auteurs ont préparé