Intrinsic Reactivities of Amino Acids towards Photoalkylation with Benzophenone − A Study Preliminary to Photolabelling of the Transmembrane Protein Glycophorin A

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A systematic study of the photoalkylation of amino acids by benzophenone, a standard photosensitive probe of biomolecules, was performed addressing for the first time chemo-, regio-, and stereoselectivities. The high reactivity of the capto-dative substituted α-carbon, particularly in glycine, could be demonstrated as well as the chemical lability of the α-coupling products. Methionine was shown to be favoured both in the sense of reactivity as well as product stability. Preliminary to a project directed towards the elucidation of the topography of glycophorin A in membranes, the present model experiments focussed on the ten amino acids that constitute the transmembrane part of this protein.

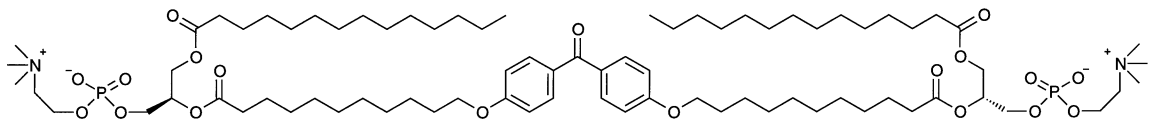
Introduction

The topography of membrane-bound proteins at atomic resolution is known only in exceptional cases.[1] This is true for glycophorin A, the protein responsible for cell recognition of human erythrocytes, although its amino acid sequence is known for twenty years and was in fact the first primary structure of a membrane protein ever elucidated.[2] In the course of our studies on the topography of biomembranes, we could demonstrate that the tandem use of the photosensitive phospholipidic transmembrane probe **1** and cholesterol (for its ordering effect) in photolabelling experiments on DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) vesicles led to regioselective functionalization of the ω-1 and ω-2 positions of surrounding phospholipidic chains and the C-25 position on the side chain of cholesterol.[3]

Having thus proven its worth, probe **1** is ready for a new ambitious goal: to elucidate the in situ topography of the transmembrane domain of membrane-bound proteins, for instance of glycophorin A. The entire process comprises (1) vesicle reconstitution using phospholipid, cholesterol, protein, and photosensitive probe, (2) photoirradiation, and (3) identification of labelled amino acid residues in the protein. As none of these steps is trivial, no chain link should be neglected. This article will focus on the purely chemical aspects of cross-linking, that is the formation of a covalent bond between the photophore and membrane constituents in proximity under photoirradiation.

As photoactivatable portion of probe **1**, we had chosen a dialkoxybenzophenone to take advantage of their selectivity and efficiency higher than those of previously used photophores.[4] In fact, benzophenone-based photolabelling has found expanded use since the mid-eighties, especially to determine receptor-ligand binding sites.[5] On the other hand, numerous biological targets under investigation by photolabelling techniques are protein-like, as is the case with glycophorin A. It is therefore surprising that basic information on the couple protein−benzophenone photophore is lacking in the literature. Only few photolabelling experiments were actually oriented toward the identification of the amino acid residues which entered into covalent binding (chemoselectivity), even without establishment of the precise binding position on the amino acid (regioselectivity).[6]

Undoubtedly, the conformation of the reactants, here the transmembrane probe **1** and glycophorin A in the phospholipidic bilayer, influence the outcome of the photoreaction, as does their location relative to each other. But it is also obvious that the specific reactivity of each type of protein-forming amino acid will influence the chemo- and the regioselectivities of the labelling process. The precise reaction mechanism not being known (rate-determining step, early



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or late transition state, etc.), any prediction or calculation remains uncertain. Nevertheless, establishing an order of intrinsic reactivities of individual amino acids against the photoactivatable probe **1** under irradiation is necessary to correctly evaluate photolabelling experiments and to help answering crucial questions like the following: Did photolabelling fail, despite a favourable arrangement of the reactants, only because the benzophenone was situated next to a low-reactive amino acid unit of the protein? Did the benzophenone moiety selectively attack one of two amino acid entities, although at the same distance and accessibility, because of its superior chemical reactivity, not of its proximity?

Even though this implies immense simplifications, the attempt is made here to systematically investigate intrinsic amino acid reactivities using low-weight model compounds in organic solution: benzophenone (**2**) or its 4,4′-dimethoxy derivative **3** will represent the photoactivatable probe, and the *N*-acetyl protected amino acid methyl esters **4** take the place of the corresponding peptide-bound amino acid residues in the protein. In fact, it was with these reaction partners that Galardy et al. demonstrated for the first time the utility of the benzophenone photophore to photolabel biomolecules, though confining themselves to one amino acid: glycine.[7] Glycophorin A is a single-pass protein with one hydrophobic region of about 20 amino acids spanning the lipid double-layer (Scheme 1).[8] As a preliminary step to the mapping of the transmembrane fragment of glycophorin A, currently under way in our laboratory, this study focusses on the 10 hydrophobic amino acids involved. However, a first step to extend it to the 20 proteinogenic amino acids is also described.

Results and Discussion

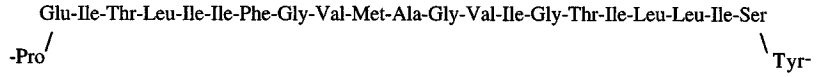
The Model

To represent amino acid residues in the peptide chain, *N*-acetyl-L-amino acid methyl esters (**4**) (Scheme 2) were chosen for the following reasons: (1) They ensure a roughly similar electronic situation; (2) their solubilities allow co-dissolution with benzophenone in organic solvents in up to molar concentrations; (3) they are easily available; (4) the acceptor-substituted methyl groups in acetyl and methyl ester should be of low reactivity against the diradicaloid, electrophilic n,π* triplet state of excited benzophenone.[9] Indeed, throughout this work, reaction at acetyl or methyl ester methyl groups was never observed. Benzophenone **2** and 4,4′-dimethoxybenzophenone **3** were used as models of the transmembrane probe **1**.

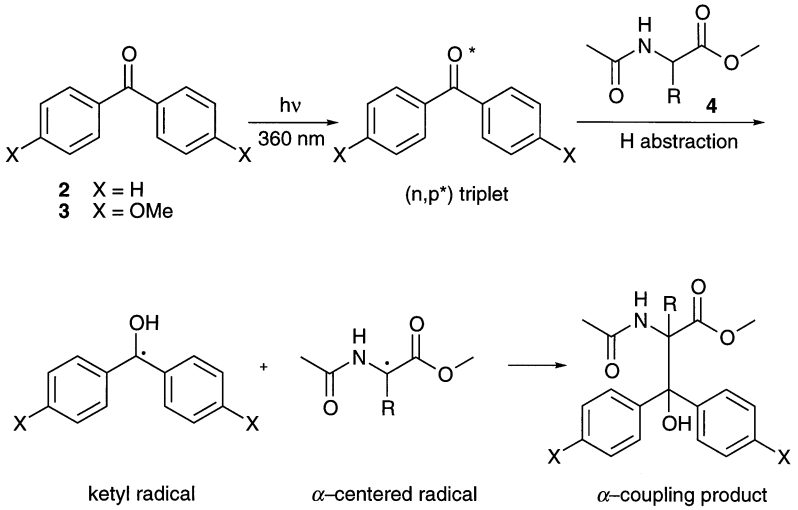
The Photochemical Reaction

Irradiation of benzophenone or of 4,4′-dimethoxybenzophenone at 350−360 nm generates a diradicaloid n,π* triplet state in which one electron from an sp^2-like n-orbital on oxygen is promoted to a π*orbital of the carbonyl group. The electron deficient oxygen n-orbital of the n,π* triplet state is the site of radical reactivity, leading to hydrogen abstraction from substrates present in a sphere with a radius of 0.23−0.31 nm centered on the ketone oxygen.[9c][9d] One possible fate of the pair of radicals thus formed is their recombination, creating a new C−C bond, this the cross-linking desired in photolabelling. Scheme 2 depicts the mechanism for the case in which H abstraction from the amino acid occurs at the α carbon.

Scheme 1. Sequence of the membrane-spanning segment of Glycophorin A



Scheme 2. The photochemical reaction



Analysis and Terminology

The term "coupling product" refers to compounds formed by recombination of one ketyl radical and one amino acid radical. The amino acid carbon bearing amino and carbomethoxy groups is called α, continuing with β-, γ-, and δ-positions on an unbranched side chain. Chemo-, regio-, and stereoselectivities elaborated in this work were quantified by yields of products formed under fixed conditions and refer to the initial amino acid derivative. All yields reported, if not stated otherwise, were calculated on the basis of the ^{1}H-NMR spectra of crude reaction products.

Comparison of Benzophenone (2) and 4,4′-Dimethoxybenzophenone (3)

The photochemical reaction between benzophenone (**2**) and *N*-acetyl glycine methyl ester (**5**) had been described by Galardy et al.[7] We reinvestigated this reaction and repeated it using 4,4′-dimethoxybenzophenone **3** as the ketone partner (Scheme 3).

Benzophenone (**2**) (0.2 mol/l) was treated with a five-fold excess of *N*-acetyl glycine methyl ester (**5**). In addition to benzopinacol **6** and coupling product **7**, already described by Galardy, the homo dimer of AcGlyOMe **8** was identified.[10] 4,4′-Dimethoxybenzophenone (**3**), in a nearly saturated solution, gave the analogous products **8**−**10**. H abstraction from one methoxy group of **3** probably initiated the formation of a second homo dimer: the keto alcohol **11**, obtained in 2.4% yield besides the known products **8** (5.6%), **9** (9.3%), and **10** (6.4%) when the proportion of ketone **3** (0.14 mol/l) was increased relative to the amino acid **5** (0.13 mol/l) (hν, benzene, 20 h, 25°C).

The yields of coupling products formed by benzophenone, **7**, and 4,4′-dimethoxybenzophenone, **10**, did not differ markedly. This is in line with comparative experiments using 2-propanol[11a] or cyclopentane[11b] as substrates. To us, it allowed modelling of the photoactivatable probe **1** by benzophenone (**2**) itself, taking advantage of its solubility in organic media which is higher than that of the structurally closer 4,4′-dimethoxybenzophenone (**3**). And as glycine turned out to be the most reactive amino acid, optimization of the reaction parameters (concentration, etc.) soon became necessary.

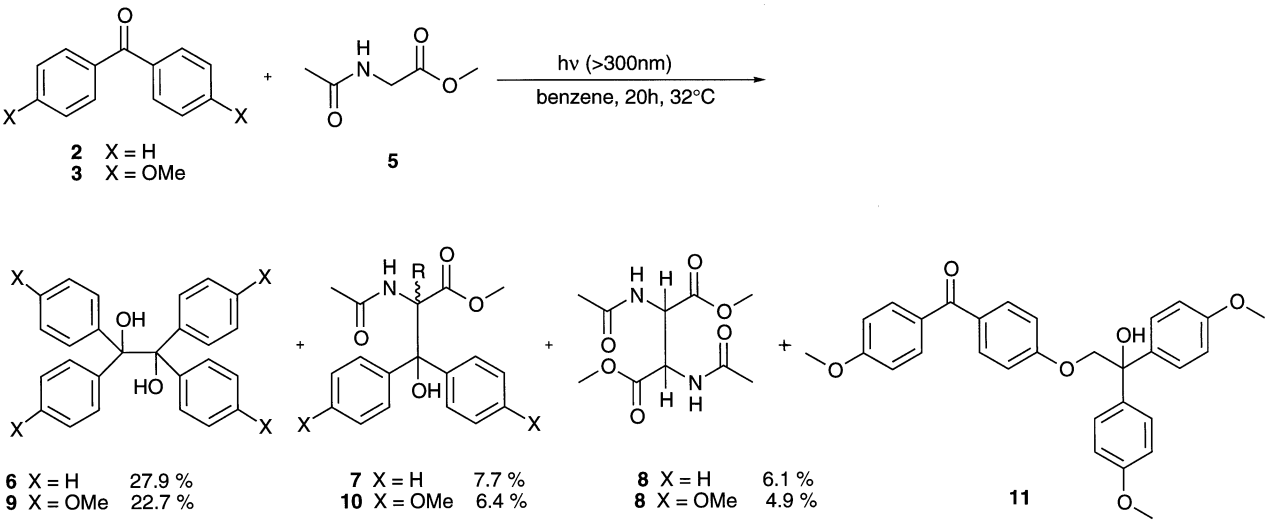
Regioselectivity

Four representative amino acids were examined, with centres of quite different predictable reactivity towards radical conditions: glycine and alanine with capto-dative substituted[12] α-centres of different substitution level and steric accessibility, phenylalanine with its benzylic position and methionine with a heteroatom. Photoreactions of all *N*-acetyl amino acid methyl esters with benzophenone were performed under the same conditions (except differences in reaction temperature), that had been optimized for methionine. All coupling products detected by thin-layer chromatography (TLC) (simultaneous UV-activity of the aromatic portion and amide staining with chlorine/*o*-tolidine) were isolated and characterized. They are depicted in Scheme 4. Reaction conditions and yields are listed in Table 1.

For glycine, only the α-coupling product **7** was found in acetonitrile. Alanine gave the α-coupling product **12** and a very low amount of ketone **13**, resulting from a radical aromatic substitution. With phenyalanine, again formation of an α-coupling product, **14**, was observed. The β-coupling product **15** was isolated only in its cyclized form **16**, by flash chromatography on silica gel. Methionine reacted selectively at the carbon atoms adjacent to sulfur, giving diastereomeric γ-coupling products **17** and **18** (absolute configurations not assigned), as well as the S-methyl coupling product **19**.

Mechanistically, the observed regioselectivities speak for the passage through the best-stabilized amino acid radicals, the transition state of the rate-determining step resembling these intermediates. Thus, the easily accessible methyl pro-

Scheme 3. Photoreaction of benzophenone **2** and 4,4′-dimethoxybenzophenone **3** on *N*-acetyl glycine methyl ester **5**



Scheme 4. Coupling products of benzophenone **2** with AcAlaOMe, AcPheOMe, AcMetOMe **12**−**19**, and dimers of AcAlaOMe **20**, **21**

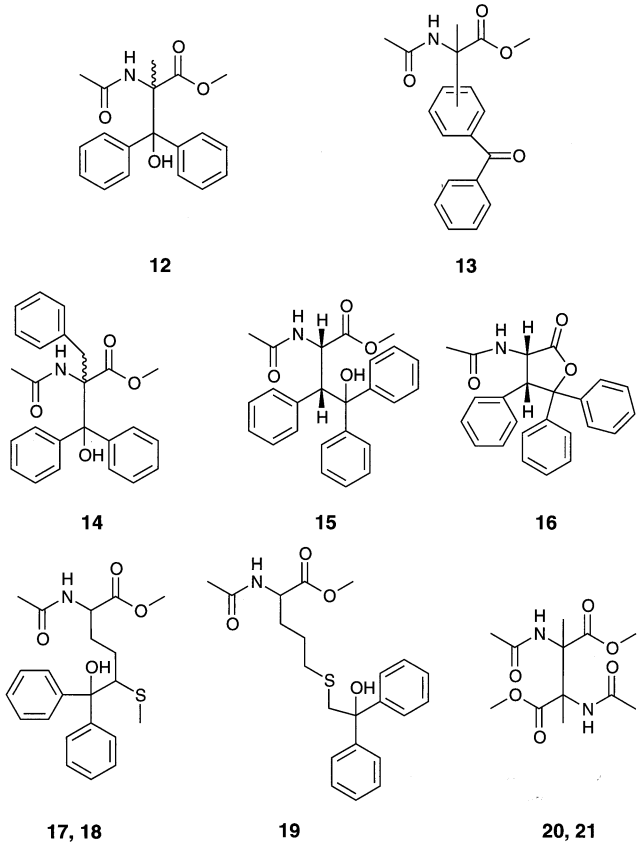


Table 1. Photoalkylation of AcGlyOMe **5**, AcAlaOMe, AcPheOMe, and AcMetOMe with benzophenone **2** in acetonitrile[a]

Substrate	Coupling product[b]	Calculated yield [%][c]	Isolated yield [%]
AcGlyOMe	**7**	24.8	19.7
AcAlaOMe	**12**	14.7	8.9[d]
	13	[e]	0.1
AcPheOMe	**14**	8.4	2.5[d]
	15	10.1	–
	16	[e]	4.2
AcMetOMe	**17**	10.5	[d,f]
	18	10.6	[d,f]
	19	8.6	[d,f]

[a] Reactant concentrations 1.0 mol/l each, reaction conditions hν, 20 h, $T = 28-37°C$. – [b] See Scheme 3 and Scheme 5. – [c] On the basis of ^{1}H-NMR spectra of crude reaction mixtures. – [d] Calculated on the basis of ^{1}H-NMR spectra of the product containing fraction(s) after the first chromatographic step. – [e] Not detected in 200-MHz ^{1}H-NMR spectrum of crude reaction mixture. – [f] Total isolated yield of coupling products **17**−**19**: 23.1%.

tons of alanine were not abstracted, but the capto-dative stabilized α-centered radical was formed exclusively. The S-methyl in methionine, little hindered and presenting three hydrogen atoms, was the site of reaction in only 29% of the total coupling product.[13] The γ-coupling products **17** and **18**, produced through a favourable secondary radical, were predominantly formed.

Isolation of the α-coupling products of AcAlaOMe **12** and AcPheOMe **14** (to a minor extent, that of AcGlyOMe **7**) was severely hampered by their high tendency to degrade to starting material, hence the poor isolated yields (Table 1). A sample of **12** was completely lost on a reversed phase RP-18 column with a CH_3CN/H_2O gradient: the sole eluates were benzophenone and AcAlaOMe. The chemical lability of the *N*-unprotected analogue of **12** and of 2-methyl serine esters in general had already been noted by Schöllkopf et al., and attributed to an easy retro-aldol degradation.[14] With all caution due to the simplicity of the model reaction under investigation, this (re)discovery might serve to judge on the stability of peptide cross-linking products during photolabelling experiments as well as on the techniques used to identify the labelled amino acid residues (typically proteolysis, purification, sequencing).[15]

In two cases, the extent of amino acid dimerization was explored by product isolation and characterization. AcGlyOMe formed the α,α′-dimer **8** (Scheme 4) (51:49 mixture of diastereomers) in a 13.3% yield, whereas the sterically more hindered AcAlaOMe dimerized in only 3.7% yield, but again regioselectively to the diastereomeric α,α′-dimers **20** and **21** (ratio 49:51, absolute configurations not assigned).

Stereoselectivity

The enantiomeric configuration of the investigated amino acids AcAlaOMe, AcPheOMe, and AcMetOMe was purely L. Formation of α-coupling products was accompanied by almost complete racemization, as shown by the absence of rotatory power and by ^{1}H-NMR in the presence of a chiral shift reagent: **12** (enantiomers 51.4:48.6), **14** (enantiomers 51.6:48.4). Evidently, planarization of the α-carbon centered radicals to allow capto-dative delocalization of the unpaired electron was faster than radical recombination. This lack of stereospecifity in radical reactions is well known.[16a]

The γ-lactone **16** was diastereomerically pure by ^{1}H-NMR examination. The coupling constant of the two ring hydrogens was 7.4 Hz, a strong evidence for a *cis* relationship.[17] An X-ray diffraction study unambigously proved this assignment. Though the enantiomeric purity of γ-lactone **16** was not proven yet, it may be assumed that the unreacted α-carbon conserved its absolute configuration. In fact, the high positive value of optical rotation ($[\alpha]^{20}$ +157 at 589 nm, +623 at 365 nm, $c = 1.0$ in MeOH) is indicative of a positive Cotton effect. While we are aware of its uncertainties as to ring conformations,[18] application of the empirical "lactone sector rule"[19] thus suggested the absolute configuration of γ-lactone **16** to be (*S*) at the α-, (*R*) at the β-carbon.

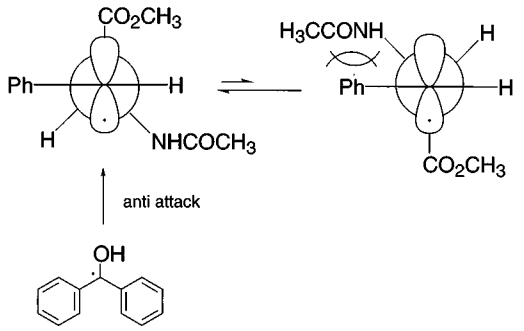
A tentative explanation of the stereoselectivity observed in the formation of γ-lactone **16** is provided by the recent review of Curran, Porter, and Giese.[16b] Scheme 5 shows how the simultaneous effects of planarization (to favour delocalization of the unpaired benzylic electron) and of the avoidance of "allylic strain" lead to a prefered conformation, an inevitable prerequisite for acyclic stereoselection. Attacking the prochiral centre anti to the largest sub-

stituent at the adjacent carbon eventually establishes diastereoselection. The assumptions implicated are:

(1) rapid hydrogen abstraction from the β-carbon of AcPheOMe by $^3(n,\pi^*)$ benzophenone should be followed by rate determining recombination passing through an early transition state that resembles the radicalic amino acid intermediate. This is in agreement with the conclusions drawn from the observed regioselectivities.

(2) The carbomethoxy group should be (and is unambiguously) the largest substituent on the α-carbon.

Scheme 5. A tentative explanation of the diastereoselection (1,2-asymmetric induction) leading to the formation of γ-lactone **16**



Chemoselectivity

The ten amino acids present in the transmembrane domain of glycophorin A were separately irradiated in the form of their *N*-acetyl *O*-methyl esters with benzophenone under the conditions which had been optimized for AcMetOMe. Table 2 lists the percentages of converted amino acid. By far the highest conversions were found for glycine, followed by methionine.

Table 2. Photoirradiation of *N*-acetyl amino acid methyl esters and benzophenone (**2**) in acetonitrile[a]

Amino acid derivative	Converted amino acid derivative [%][b]
AcGlyOMe	51 (25)
AcMetOMe	40 (30)
AcThrOMe	27
AcLeuOMe	26
AcPheOMe	25 (19)
AcValOMe	25
AcAlaOMe	22 (15)
AcIleOMe	19
AcSerOMe	12
AcGluOMe	1

[a] Reactant concentrations 1.0 mol/l each, reaction conditions hν, 20 h, $T = 32$°C. – [b] Calculated on the basis of ^{1}H-NMR spectra of crude reaction mixtures (error margin ±2%), in brackets total yields of coupling products formed.

The outstanding reactivity of glycine, and its readiness to undergo hydrogen abstraction from the α-carbon, had been recognized and intensively exploited by Elad et al.[20] to introduce new side chains into amino acids, peptides, and proteins by photoalkylation. In a more mechanistically oriented study, Easton[21] investigated chemo- and regioselectivities of amino acids against radical bromination. The high reactivity of glycine again observed was attributed to optimal delocalization of the unpaired electron in the radical intermediate, while all amino acids bearing a side-chain are forced into a compromise between electronically favourable planarization and sterically imposed pyramidalization.

A first step was made towards an extension of the study to the 20 proteinogenic amino acids. The percentages of converted amino acids are listed in Table 3.

Table 3. Photoirradiation of *N*-acetyl amino acid methyl esters and benzophenone (**2**) in pyridine/water, 4:1[a]

Amino acid derivative	Converted amino acid derivative [%][b]
AcProOMe	53
AcMetOMe	45 (31)
AcThrOMe	38
AcArgOMe·HCl	33
AcSerOMe	29
AcLeuOMe	25
AcTyrOMe·H_2O	21
AcTrpOMe	16
AcGlyOMe	16 (8)
AcHisOMe·HOAc	14
AcLysOMe·HCl	12
AcIleOMe	11
AcPheOMe	9 (9)[c]
AcValOMe	6
$(AcCysOMe)_2$	5
AcAlaOMe	1 (0.3)
AcAsnOMe	0
AcAspOMe	0
AcGlnOMe	0
AcGluOMe	0

[a] Reactant concentrations 0.5 mol/l each, reaction conditions hν, 20 h, $T = 37$°C. – [b] Calculated on the basis of ^{1}H-NMR spectra of crude reaction mixtures (error margin ±2%), in brackets total yields of coupling products formed. – [c] Only β-coupling products (92.5% γ-lactone).

To find a common, photochemically stable solvent for all reactants, which differ markedly in their hydrophilicity, was a delicate problem, eventually solved by choosing a pyridine/water mixture. It was interesting to look at what happens in the new solvent to the reactivity of those amino acids that were already studied in acetonitrile (Table 2). Three groups can be distinguished: Alanine, glycine, isoleucine, phenylalanine, and valine showed lower conversions, leucine and methionine almost the same, whereas serine and threonine were more reactive in pyridine/water. The latter observation may be attributed to a solvent effect facilitating hydrogen abstraction in a hydrophilic medium, which for alcohols is known to proceed through polar transition states.[22] On the other hand, it is certainly a secondary reaction, the base-catalyzed retro-degradation of photochemically formed α-coupling products, that accounts for the decrease (almost to zero for alanine) of the reactivity of the first group of amino acids. The chemical lability of α-coupling products has already been demonstrated in the cases of alanine, phenylalanine and, to a minor extent, of glycine. This interpretation is further confirmed by the fact that methionine, known not to form an α-coupling product, roughly maintained its degree of conversion in both reaction media. Similarly to alanine, only traces of α-coupling product have been detected with phenylalanine: the yield

given in Table 3 refers to the β-coupling product exclusively which, in acetonitrile entirely present in its ring open state, appeared in pyridine/water cyclized to γ-lactone **16** in 92.5% yield. A non-reactive common solvent for the 20 amino acids remains to be found.

Despite its limitations, the chemoselectivity order given in Table 3 provides useful information: the high to moderate activation exerted by a nitrogen atom in the amino acid side-chains of arginine and lysine[22] and the quasi-inertness of compounds containing an amide or carboxyl function (asparagine, aspartic acid, glutamine, glutamic acid). The high reactivity of proline is mostly to be remarked: it takes the head of the field. Certainly the high rigidity of the cyclic amino acid sterically promotes attack. The regioselectivity of product formation was not established here. An α-coupling product is not a prori favoured.[21b][23] However, the occurence of 1-acetyl-4,5-dihydro-pyrrole-2-carboxylic acid methyl ester,[24] the product of an elimination reaction discussed for proteins,[5] could be excluded by the absence of the relevant ^{1}H-NMR signals in the crude reaction mixture.

Conclusion

Regioselectivity studies on four representative amino acids towards photoalkylation by benzophenone gave an insight into the high tendency to form α-coupling products, as well as the chemical lability of these compounds, a hint for complications which might afflict the detection of photolabelling on peptide backbones. For the first time, an order of relative chemical reactivities was established for ten lipophilic amino acids frequently present in the hydrophobic region of membrane bound proteins, towards benzophenone, a widely used photophore in cross-linking experiments. The ideal hydrophobic general reagent would label only, but all, amino acid residues in direct contact with the lipid bilayer.[15] Far from being this ideal reagent, benzophenone appeared sensitive to a wide range of reactivities inherent to the structures of the ten amino acids under investigation, which in turn justifies 4 the present study. Methionine emerged as outstanding for its reactivity and for the chemical stability of the cross-linked products once formed.

It is to be noted that the yields obtained in the present study are rather low, when compared to those previously obtained with phospholipids and the membrane-bond probes.[3][4] This is obviously linked with the fact that we are dealing with bimolecular reactions, in the present case dependent on the concentrations of both partners in solution, whereas in a membrane the probe is constantly in direct contact with the neighbouring phospholipid chains.

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Experimental Section

Materials: N-acetyl amino acid methyl esters were prepared according to literature procedures (AcAspOMe, AcGluOMe,[25] AcGlyOMe[26]) or purchased from Bachem. Benzophenone and 4,4′-dimethoxybenzophenone were obtained from Acros. The reaction solvents used were benzene (Normapur, analytical grade), acetonitrile (sds, HPLC grade), pyridine (Fluka), and water (Millipore quality).

Equipment: Melting points were measured on a Reichert hot stage microscope and are reported uncorrected. − UV-spectra were measured in MeOH on a Beckman DU-7 spectrophotometer. − IR-spectra were determined with a Perkin-Elmer 1600 series FTIR spectrometer. − NMR-spectra were recorded on Bruker WP 200 or AM 400 spectrometers (measuring frequency and solvent in brackets). Chemical shifts are reported in ppm on the δ-scale relative to $CDCl_3$ (δ = 7.26), C_6D_6 (δ = 7.20), or CD_3OD (δ = 3.35) for ^{1}H-NMR spectra, to $CDCl_3$ (δ = 77.0), C_6D_6 (δ = 128.0), or CD_3OD (δ = 49.0) for ^{13}C-NMR spectra. ^{1}H-NMR data are presented in the following manner: multiplicity (abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak), coupling constant(s) in hertz, number of protons, and assignment. DEPT experiments served to reveal the subtitution pattern of ^{13}C-NMR signals. H/D exchange experiments with D_2O confirmed the assignment of hydroxylic protons. − Mass spectra were measured on a VG Analytical ZAB-HF apparatus in the FAB mode, using *m*-nitrobenzylic alcohol as matrix substance. − Optical rotations were measured on a Perkin-Elmer 241 polarimeter. − Elemental analyses were performed by the Strasbourg Division of the Service Central de Microanalyse of CNRS. − High pressure liquid chromatography was done with the following set-up: Rheodyne injector 7125, Waters 600E multisolvent delivery system, Waters 486 tunable absorbance detector, Waters 746 data module, Gilson FC 203B fraction collector. The columns used were Waters Delta Pak 15μ C18 100Å size 7.8×300 mm, run at 3.0 ml/min for reversed phase (RP) HPLC, and Zorbex Sil 9.4 mm ID×25 cm (5 μm) run at 5.0 ml/min for normal phase (NP) HPLC. Thin-layer chromatography (TLC) for analysis of reactions and fractions was performed on Merck 60 F_{254} silica gel plates; compounds were visualized by UV or staining: solvent was blown off, then chromatograms treated with chlorine for 10 min, excess chlorine was blown off within 15 min and TLC plates dipped into a solution of *o*-toluidine (15 g) (HIGHLY TOXIC, suspected to be CARCINOGENIC!), in *tert*-butyl methyl ether (270 ml) and glacial acetic acid (30 ml). Flash chromatography (0.3−0.5 bar) was carried out using Merck Si 60 silica gel of 0.015−0.04 mm particle size.

General Procedure for the Photoalkylation of N-Acetyl Amino acid Methyl Esters: Solvents were deoxygenated by argon bubbling for 1 h. Reactions were carried out under argon in Pyrex tubes (4.2 mm inner diameter), arranged circumferentially around the cooling jacket (Pyrex) of a MAF 250-W high-pressure mercury-vapour lamp (UV PROTECTION! Cut-off by Pyrex around 300 nm; most intense mercury lines at 366, 405, 436, 546 nm). The distance between the tube and the lamp centre was about 35 mm. To measure the reaction temperature, a sensor was installed in one of the tubes containing pure solvent. Without external thermostatting, by simply using a fast cooling water flow, the reaction temperature was stabilized in the useful range of 28−37°C, well above the phase transition temperature of DMPC (24°C). Thus the model experiment met the conditions envisaged for cross-linking of transmembrane protein glycophorin A in reconstituted DMPC vesicles. Routinely, acetonitrile was used as solvent, as it dissolves the *N*-acetyl methyl ester derivatives of the ten amino acids present in the glycophorin A transmembrane fragment as well as benzophenone at molar concentrations. Standard reactant concentrations (1.0 mol/l) and irradiation times (20 h) were those found optimal for AcMetOMe. Under these conditions 63.6% of initial benzo-

phenone were converted. Higher or lower concentrations as well as shorter reaction times reduced the yield of coupling products formed (Table 4).

Table 4. Optimization of reactant concentration and irradiation time at the photoalkylation of AcMetOMe with benzophenone **2**[a]

Reactant concentrations [mol/l]	Irradiation time [h]	Converted AcMetOMe [%][b]
1.0	1	16
1.0	6	37
1.0	20	42
0.8	20	35
2.8	20	26

[a] Reactants in equimolar amounts, $T = 27°C$. – [b] Calculated on the basis of ^{1}H-NMR spectra of crude reaction mixtures.

Photoalkylation of AcGlyOMe **5** *by 4,4′-Dimethoxybenzophenone* (**3**): Exceptionally, this experiment was carried out in an immersion well reactor. *N*-acetyl glycine methyl ester (**5**) (5.23 g, 39.8 mmol) and 4,4′-dimethoxybenzophenone (**3**) (10.42 g, 43 mmol) were dissolved in benzene (300 ml). The reaction mixture was freed from dissolved oxygen by an argon flow over a period of 10 min by means of a Teflon tube entering into the reactor, then maintained under argon, thermostatted using a water bath, and stirred with a magnetic bar. After 2 h, 20 h, and 76 h of irradiation, samples were taken and shown by ^{1}H NMR to contain 1.1%, 6.4%, and 8.6% of coupling product **10**. After 76 h, the formerly colourless reaction mixture was yellow and a white solid, insoluble in $CHCl_3$, covered the reactor interior walls. These were hints for fulvene formation and polymerization,[27] hampering reaction progress by absorption and dimming. This observation additionally directed towards acetonitrile as the routine solvent. The reaction mixture was concentrated to give 15.33 g of a yellowish brown solid. Flash chromatography (gradient from ethyl acetate/*n*-hexane, 1:3, to pure ethyl acetate) allowed isolation of coupling product **10** (1.14 g, 7.7%). Submitting less polar fractions to reversed phase RP-HPLC (CH_3CN/H_2O gradient from 10:90 to 90:10 within 80 min, UV-detection at $\lambda = 220$ nm) separated analytical samples of tetramethoxybenzopinacol **9**[28] (yield 6.5% by ^{1}H NMR) and keto alcohol **12** (yield 2.4% by ^{1}H NMR). Amino acid dimer **8**[10] (yield 6.9% by ^{1}H NMR) was isolated with benzophenone as photoalkylation reagent (see below).

Coupling Product **10:** White solid (ethyl acetate); M.p. 148°C. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.36 (m, 4 H, aryl-H), 6.82 (m, 4 H, aryl-H), 6.35 [d, br, ^3J(H,H) = 8.5 Hz, 1 H, NH], 5.57 [d, ^3J(H,H) = 8.5 Hz, 1 H, CH], 4.33 (s, 1 H, OH), 3.76 (s, 3 H, aryl-OCH_3), 3.75 (s, 3 H, aryl-OCH_3), 3.51 (s, 3 H, CO_2CH_3), 1.88 (s, 3 H, $COCH_3$). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.0, 170.1, 158.8, 158.7, 136.2, 134.9, 126.7, 126.5, 113.7, 78.8, 57.2, 55.2, 52.4, 22.9. – IR (KBr): $\tilde{\nu}$ = 3459, 3375, 3081, 3006, 2954, 2931, 2905, 2833, 1714, 1675, 1609, 1583, 1510, 1249, 1182, 1034, 812 cm^{-1}. – UV (MeOH): λ_{max} = 282, 274, 232 nm. – MS (FAB): *m/z* (%): 374 (3) $[M+H]^+$, 356 (51), 324 (61), 243 (100). – $C_{20}H_{23}NO_6$ (373.4): calcd C 64.33, H 6.21, N 3.75; found C 64.6, H 6.3, N 3.5.

Keto Alcohol **11:** White solid; M.p. 97–98°C. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.77 (m, 4 H, aryl-H), 7.36 (m, 4 H, aryl-H), 6.96 (m, 4 H, aryl-H), 6,87 (m, 4 H, aryl-H), 4.48 (s, 2 H, OCH_2), 3.88 (s, 3 H, aryl-OCH_3), 3.80 (s, 6 H, 2× aryl-OCH_3), 3.28 (s, 1 H, OH). – ^{13}C NMR (50 MHz, C_6D_6): δ = 193.5, 163.2, 161.8, 159.5, 137.0, 132.5, 132.4, 132.1, 131.4, 130.5, 114.6, 113.9, 113.8, 112.9, 77.4, 74.7, 54.9. – IR (KBr): $\tilde{\nu}$ = 3472, 3039, 3001, 2933, 2836, 1645, 1601, 1509, 1461, 1418, 1305, 1250, 1167, 1032, 928, 833 cm^{-1}. – UV (MeOH): λ_{max} = 294, 283, 227, 208 nm. – MS (FAB): *m/z* (%): 485 (37) $[M+H]^+$, 467 (13), 243 (100). – $C_{30}H_{28}O_6$ (484.5): calcd C 74.36, H 5.82; found C 74.4, H 5.7.

Photoalkylation of AcGlyOMe **5** *by Benzophenone* (**2**): According to the general procedure, *N*-acetyl glycine methyl ester (**5**) (5.245 g, 40 mmol) and benzophenone (**2**) (7.289 g, 40 mmol) were dissolved in acetonitrile to give 40 ml of a clear, colourless solution which was distributed to Pyrex tubes and irradiated (20 h, $T = 28°C$). After evaporation of the solvent, 12.303 g of a clear, yellow, viscous oil were obtained. Flash chromatography (CH_2Cl_2/MeOH gradient from 100:1 to 20:1) yielded α-coupling product **7** (2.465 g, 19.7%) and AcGlyOMe dimer **8** (1.143 g, 11.0%), the latter as an unseparated mixture of diastereomers. Yields calculated on the basis of the ^{1}H-NMR spectrum of the crude reaction mixture were: benzopinacol (**6**) (15.3%), coupling product **7** (24.8%), amino acid dimer **8** (13.3%).

Coupling Product **7:** White solid (ethyl acetate). – M.p. 173–174°C. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.51–7.17 (m, 10 H, aryl-H), 6.37 [d, br, ^3J(H,H) = 8.5 Hz, 1 H, NH], 5.68 [d, ^3J(H,H) = 8.5 Hz, 1 H, CH], 4.46 (s, 1 H, OH), 3.50 (s, 3 H, CO_2CH_3), 1.87 (s, 3 H, $COCH_3$). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.9, 170.1, 143.7, 142.4, 128.5, 127.6, 127.5, 125.5, 125.2, 79.3, 57.2, 52.4, 22.8. – IR (KBr): $\tilde{\nu}$ = 3449, 3339, 3280, 3056, 3004, 2954, 1747, 1733, 1654, 1534, 1439, 1449, 1433, 1373, 1206, 1174, 745, 702 cm^{-1}. – UV (MeOH): λ_{max} = 258, 253, 231 nm. – MS (FAB): *m/z* (%): 314 (48) $[M+H]^+$, 296 (99), 264 (100). – $C_{18}H_{20}NO_4$: calcd 314.1392; found 314.1389 (MS).

AcGlyOMe Dimer **8:** White solid; M.p. 125–133°C. – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 6.90 [d, br, ^3J(H,H) = 6.3 Hz, 0.51×1 H, NH], 6.36 [d, ^3J(H,H) = 7.4 Hz, 0.49×1 H, NH], 5.04 (m, 2 H, CHCH), 3.81 (s, 0.51×3 H, CO_2CH_3), 3.78 (s, 0.49×3 H, CO_2CH_3), 2.07 (s, 0.51×3 H, $COCH_3$), 2.02 (s, 0.49×3 H, $COCH_3$). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.3, 170.4, 170.0, 169.2, 55.0, 53.7, 53.0, 22.9, 22.7. – IR (KBr): $\tilde{\nu}$ = 3252, 3065, 3010, 2958, 2849, 1744, 1667, 1550, 1436, 1378, 1339, 1305, 1266, 1244, 1214, 1169, 1130 cm^{-1}. – UV (MeOH): λ_{max} = 214 nm. – MS (FAB): *m/z* (%): 261 (100) $[M+H]^+$, 219 (16), 159 (13). – $C_{10}H_{17}N_2O_6$: calcd 261.1087; found 261.1079 (MS).

Photoalkylation of AcAlaOMe by Benzophenone: Following the general procedure, *N*-acetyl alanine methyl ester (4.354 g, 30 mmol) and benzophenone (**2**) (5.467 g, 30 mmol) were dissolved in acetonitrile to give 30 ml of a clear, colourless solution which was distributed to Pyrex tubes and irradiated (20 h, $T = 37°C$). After evaporation of the solvent, 9.553 g of a clear, yellow oil were obtained. Flash chromatography running a CH_2Cl_2/toluene/MeOH gradient from 50:50:1 to 1:0:1 gave α-coupling product **12** (613 mg, contents 71.4% by ^{1}H NMR) accompanied by starting material benzophenone (22.6% by ^{1}H NMR) and AcAlaOMe (6.0% by ^{1}H NMR). An analytically pure sample of **12** was obtained by repeated flash chromatography (CH_2Cl_2). Only one minor coupling product was detected in the crude reaction mixture by TLC. Submitting the relevant fraction from the initial flash chromatography to RP-HPLC (gradient CH_3CN/H_2O 10:90 to 90:10 within 80 min, UV-detection at $\lambda = 220$ nm) allowed isolation of ketone **13** (10.5 mg, 0.1% isolated yield). The most polar fraction obtained in the nitial flash chromatography was further separated by HPLC on silicagel (1.4-dioxane/*n*-heptane, 3:7, + 0.1% H_2O, UV-detection at $\lambda = 220$ nm), giving analytically pure diastereomeric amino acid dimers **20** and **21**. Yields calculated on the basis of an ^{1}H-NMR spectrum of the crude reaction mixture were: benzopinacol **6** (4.5%), α-coupling

product **12** (14.7%), ketone **13** (not detectable), amino acid dimers **20** (1.8%), and **21** (1.9%).

α-Coupling Product **12:** White solid foam; M.p. 40−42°C; $[\alpha]_D^{20}$ = +0.2 (*c* = 1.0 in $CHCl_3$). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.68 (m, 2 H, aryl-H), 7.43 (m, 2 H, aryl-H), 7.28 (m, 6 H, aryl-H), 6.98 (s, br, 1 H, NH), 6.37 (s, br, 1 H, OH), 3.38 (s, 3 H, CO_2CH_3), 1.82 (s, 3 H, $COCH_3$ or CCH_3), 1.80 (s, 3 H, $COCH_3$ or CCH_3); addition of chiral shift reagent europium tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate], (+)-Eu(hfc)$_3$, allowed determination of the enantiomeric ratio as 51.4:48.6. − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 175.1, 170.7, 144.7, 142.9, 127.7, 127.3, 82.4, 68.8, 52.8, 23.6, 19.7. − IR (KBr): $\tilde{\nu}$ = 3396, 3058, 3025, 2950, 1736, 1664, 1600, 1494, 1449, 1376, 1304, 1260, 1157, 757, 704 cm^{-1}. − UV (MeOH): λ_{max} = 253, 227 nm. − MS (FAB): *m/z* (%): 328 (65) $[M+H]^+$, 310 (100), 268 (24), 208 (23), 183 (72), 146 (72). − $C_{19}H_{22}NO_4$ calcd 328.1549; found 328.1550 (MS).

Ketone **13:** Yellow oil. − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.90−7.30 (m, 9 H, aryl-H), 7.00 (s, br, 1 H, NH), 3.72 (s, 3 H, CO_2CH_3), 2.09 (s, 3 H, $COCH_3$ or CCH_3), 2.04 (s, 3 H, $COCH_3$ or CCH_3). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.1, 173.4, 168.8, 144.5, 137.4, 132.5, 130.3, 130.0, 128.3, 125.9, 62.0, 53.5, 23.9, 22.1. − IR (film): $\tilde{\nu}$ = 3304, 3059, 3000, 2951, 1741, 1659, 1606, 1578, 1530, 1448, 1280, 1252, 924, 732, 703 cm^{-1}. − UV (MeOH): λ_{max} = 256, 208 nm. − MS (FAB): *m/z* (%): 326 (100) $[M+H]^+$, 267 (54), 224 (30). − $C_{19}H_{20}NO_4$ calcd 326.1392; found 326.1397 (MS).

Amino Acid Dimer **20:** White solid; M.p. 155−157°C; $[\alpha]_D^{20}$ = 0.0 (*c* = 1.0 in $CHCl_3$). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.44 (s, br, 2 H, 2×NH), 3.82 (s, 6 H, 2×CO_2CH_3), 2.01 (s, 6 H, 2×$COCH_3$), 1.46 (s, 6 H, 2×CCH_3). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 169.8, 62.3, 53.0, 23.2, 18.0. − IR (KBr): $\tilde{\nu}$ = 3268, 3199, 3077, 3007, 2955, 2851, 1751, 1732, 1657, 1545, 1449, 1376, 1307, 1243, 1095 cm^{-1}. − UV (MeOH): λ_{max} = 206 nm. − MS (FAB): *m/z* (%): 289 (36) $[M+H]^+$, 257 (22), 229 (14), 146 (100). − $C_{12}H_{20}N_2O_6$ (288.3): calcd C 49.99, H 6.99, N 9.72; found C 49.8, H 6.8, N 9.5.

Amino Acid Dimer **21**: Yellowish white, highly viscous oil; $[\alpha]_D^{20}$ = −1.4 (*c* = 1.0 in MeOH). − ^{1}H NMR (200 MHz, CD_3OD): δ = 3.72 (s, 6 H, 2×CO_2CH_3), 2.02 (s, 6 H, 2×$COCH_3$), 1.68 (s, 6 H, 2×CCH_3). − ^{13}C NMR (100 MHz, CD_3OD): δ = 173.3, 172.5, 65.4, 53.2, 22.9, 18.5. − IR (KBr): $\tilde{\nu}$ = 3386, 3008, 2958, 1728, 1670, 1530, 1410, 1362, 1268, 1094 cm^{-1}. − UV (MeOH): λ_{max} = 205 nm. − MS (FAB): *m/z* (%): 289 (10) $[M+H]^+$, 231 (100), 183 (36). − $C_{12}H_{20}N_2O_6$ (288.3): calcd C 49.99, H 6.99, N 9.72; found C 50.3, H 6.9, N 9.5.

Photoalkylation of AcPheOMe by Benzophenone (**2**): According to the general procedure *N*-acetyl phenylalanine methyl ester (8.850 g, 40 mmol) and benzophenone (**2**) (7.289 g, 40 mmol) were dissolved in acetonitrile to give 40 ml of a clear, colourless solution which was distributed to Pyrex tubes and irradiated (20 h, *T* = 32°C). After evaporation of the solvent 14.740 g of a yellow paste were obtained. Flash chromatography running a gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (100:1) allowed separation of two impure coupling products **14** (565 mg, contents 54.8% by ^{1}H NMR, rest benzophenone), and **16** (641 mg, contents 94.5% by ^{1}H NMR, rest AcPheOMe). Analytically pure samples were isolated by repeated flash chromatography eluting with CH_2Cl_2/*n*-hexane, 1:1 (**14**), or CH_2Cl_2/MeOH, 100:1 (**16**). Yields calculated on the basis of a ^{1}H-NMR spectrum of the crude reaction mixture were: benzopinacol (**6**) (10.4%), α-coupling product **14** (8.4%), and β-coupling product **15** (10.1%).

α-Coupling Product **14:** White solid foam ; M.p. 119°C; $[\alpha]_D^{20}$ = +0.1 (*c* = 1.0 in MeOH). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.75-6.85 (m, 16 H, 15aryl-H, NH), 4.20 [d, ^2J(H,H) = 13.9 Hz, 1 H, benzylic H], 3.45 [d, ^2J(H,H) = 13.9 Hz, 1 H, benzylic H], 3.30 (s, 3 H, CO_2CH_3), 1.73 (s, 3 H, $COCH_3$), 1.56 (s, 1 H, OH); addition of chiral shift reagent (+)-Eu(hfc)$_3$, allowed determination of the enantiomeric ratio as 51.6:48.4. − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.3, 171.6, 145.5, 143.8, 136.2, 132.5, 130.3, 130.1, 128.4, 128.2, 127.8, 127.6, 127.4, 127.1, 127.0, 83.8, 75.4, 52.4, 35.2, 23.7. − IR (KBr): $\tilde{\nu}$ = 3294, 3134, 3058, 3031, 2952, 1731, 1623, 1530, 1500, 1439, 1323, 1216, 758, 700 cm^{-1}. − UV (MeOH): λ_{max} = 253, 224 nm. − MS (FAB): *m/z* (%): 404 (43) $[M+H]^+$, 386 (31), 222 (100), 183 (55), 178 (52). − $C_{25}H_{25}NO_4$ (403.5): calcd C 74.42, H 6.25, N 3.47; found C 74.3, H 6.3, N 3.6.

γ-Lactone **16:** White solid (ethyl acetate); M.p. 108−110°C; $[\alpha]_D^{20}$ = +157.5 (*c* = 1.0 in MeOH). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.67−6.94 (m, 15 H, aryl-H), 5.42 [d, br, ^3J(H,H) = 7.4 Hz, 1 H, NH], 5.11 [tr, ^3J(H,H) = 7.4 Hz, 1 H, NCH], 4.90 [d, ^3J(H,H) = 7.4 Hz, 1 H, benzylic H], 1.76 (s, 3 H, $COCH_3$); a second ^{1}H-NMR spectrum measured in C_6D_6 affirmed diastereomeric purity. − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.2, 170.0, 142.8, 140.8, 134.5, 129.3, 129.2, 128.7, 128.3, 128.1, 127.7, 127.1, 125.5, 124.9, 91.3, 55.2, 54.5, 53.5, 22.6. − IR (KBr): $\tilde{\nu}$ = 3275, 3061, 3035, 2909, 1782, 1654, 1604, 1540, 1495, 1450, 1374, 1171, 969, 743, 700 cm^{-1}. − UV (MeOH): λ_{max} = 259, 231 nm. − MS (FAB): *m/z* (%): 372 (100) $[M+H]^+$, 313 (28), 267 (39), 161 (26). − $C_{24}H_{22}NO_3$ calcd 372.1600; found 372.1602 (MS).

Photoalkylation of AcMetOMe by Benzophenone (**2**): Following the general procedure, *N*-acetyl methionine methyl ester (4.004 g, 19.5 mmol) and benzophenone (**2**) (3.553 g, 19.5 mmol) were dissolved in acetonitrile to give 19.5 ml of a clear, colourless solution which was distributed to Pyrex tubes and irradiated (20 h, *T* = 30°C). After evaporation of the solvent 7.217 g of a clear, yellow, highly viscous oil were obtained. Flash chromatography (gradient: ethyl acetate/*n*-hexane from 1:1 to 3:2) removed benzophenone (**2**), benzopinacol (**6**), and in part AcMetOMe. A less polar product fraction was submitted to HPLC on silicagel (eluent 1.4-dioxane/*n*-heptane, 1:4, + 0.1% H_2O, UV detection at λ = 254 nm) separating the diastereomeric γ-coupling products **17** and **18** in analytically pure form. Removal of excess AcMetOMe from a more polar product fraction using RP-HPLC (gradient CH_3CN/H_2O 10:90 to 90:10 within 80 min, UV-detection at λ = 220 nm) allowed isolation of the pure S-methyl coupling product **19**. Yields calculated on the basis of a ^{1}H-NMR spectrum of the crude reaction mixture were: benzopinacol (**6**) (16.8%), γ-coupling products **17** (10.5%) and **18** (10.6%), and S-methyl coupling product **19** (8.6%). After the first flash chromatography, a total of 23.1% of coupling products **17**−**19** was present in the product fractions by ^{1}H NMR.

γ-Coupling Product **17:** White solid; M.p. 57°C; $[\alpha]_D^{20}$ = +0.3 (*c* = 1.0 in MeOH). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.60−7.12 (m, 10 H, aryl-H), 6.06 [d, br, ^3J(H,H) = 8.8 Hz, 1 H, NH], 5.09 [ddd, ^3J(H,H) = 8.8, 8.8, 3.6 Hz, 1 H, NCH], 3.78 [dd, ^3J(H,H) = 10.1, 2.7 Hz, 1 H, SCH], 3.71 (s, 3 H, CO_2CH_3), 3.40 (s, 1 H, OH), 2.12 (s, 3 H, $COCH_3$ or SCH_3), 1.96−1.70 (m, 2 H, CH_2), 1.76 (s, 3 H, $COCH_3$ or SCH_3). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.0, 170.4, 146.1, 144.5, 128.4, 128.2, 127.1, 125.9, 125.6, 80.5, 56.3, 52.6, 50.9, 36.4, 23.3, 17.4. − IR (KBr): $\tilde{\nu}$ = 3362, 3058, 3033, 2951, 2922, 2855, 1740, 1668, 1598, 1541, 1493, 1448, 1373, 1245, 747, 704 cm^{-1}. − UV (MeOH): λ_{max} = 259, 227 nm. − MS (FAB): *m/z* (%): 388 (3) $[M+H]^+$, 370 (100), 311 (14), 263 (17), 251 (28), 204 (80), 183 (53). − $C_{21}H_{26}NO_4$ calcd 388.1583; found 388.1554 (MS).

γ-Coupling Product **18:** White solid; M.p. 116°C; $[\alpha]_D^{20} = +13.0$ (c = 1.0 in MeOH). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.79–7.51 (m, 4 H, aryl-H), 7.24-6.86 (m, 6 H, aryl-H), 6.06 [d, br, ^3J(H,H) = 7.4 Hz, 1 H, NH], 5.08 [ddd, ^3J(H,H) = 8.3 Hz, 7.4 Hz, 5.4 Hz, 1 H, NCH], 3.91 [dd, ^3J(H,H) = 10.4 Hz, 2.1 Hz, 1 H, SCH], 3.49 (s, 1 H, OH), 3.29 (s, 3 H, CO_2CH_3), 2.29 [ddd, ^2J(H,H) = 14.8 Hz, ^3J(H,H) = 8.3 Hz, 2.1 Hz, 1 H, methylene H], 1.74 [ddd, ^2J(H,H) = 14.8 Hz, ^3J(H,H) = 10.4 Hz, 5.4 Hz, 1 H, methylene H], 1.49 (s, 3 H, $COCH_3$ or SCH_3), 1.44 (s, 3 H, $COCH_3$ or SCH_3). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.7, 169.9, 145.9, 144.4, 128.4, 128.2, 127.1, 126.0, 125.8, 80.7, 55.1, 52.4, 51.5, 34.8, 23.1, 17.1. – IR (KBr): $\tilde{\nu}$ = 3494, 3239, 3059, 2950, 2920, 2846, 1731, 1635, 1600, 1560, 1493, 1448, 1020, 751, 706 cm^{-1}. – UV (MeOH): λ_{max} = 258, 222 nm. – MS (FAB): *m/z* (%): 388 (3) $[M+H]^+$, 370 (100), 311, 251 (34), 204 (85), 183 (55). – $C_{21}H_{26}NO_4$ calcd 388.1583; found 388.1577 (MS).

S-Methyl Coupling Product **19:** Clear, colourless, highly viscous oil; $[\alpha]_D^{20} = -5.6$ (c = 1.0 in MeOH). – ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.50–7.18 (m, 10 H, aryl-H), 6.03 [d, br, ^3J(H,H) = 7.4 Hz, 1 H, NH], 4.69 (m, 1 H, NCH), 3.79 (s, 1 H, OH), 3.71 (s, 3 H, CO_2CH_3), 3.42 [s, 2 H, $SCH_2C(OH)Ph_2$], 2.48 (m, 2 H, SCH_2CH_2), 2.18–1.80 (m, 2 H, SCH_2CH_2), 1.99 (s, 3 H, $COCH_3$). – ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.7, 170.2, 145.7, 128.6, 128.3, 127.3, 126.2, 77.5, 52.6, 51.4, 46.2, 32.6, 30.1, 23.1. – IR (KBr): $\tilde{\nu}$ = 3304, 3060, 3032, 2952, 2848, 1740, 1669, 1599, 1544, 1448, 1374, 1220, 1171 cm^{-1}. – UV (MeOH): λ_{max} = 253, 229 nm. – MS (FAB): *m/z* (%): 388 (5) $[M+H]^+$, 370 (100), 251 (21), 205 (28), 190 (32), 183 (53), 158 (92). – $C_{21}H_{25}NO_4$ (387.5): calcd C 65.09, H 6.50, N 3.61; found C 65.43, H 6.55, N 3.53.

Chemoselectivity: The Pyrex walls of the lamp′s cooling jacket are supposed to filter off wavelengths below 300 nm, which would be destructive to amino acids.[29] Nevertheless, blank experiments were performed irradiating protected amino acids without benzophenone under standard conditions. The ^{1}H-NMR spectra after irradiation were identical with those of original probes, except for cystine in pyridine/H_2O, which was found to be monomerized to cysteine in 4% yield.

Ten tubes with the derivatized amino acids of Table 2 (1.0 mol/l) and benzophenone **2** (1.0 mol/l) in acetonitrile were irradiated at the same time following the general procedure (20 h, T = 32°C). In the same way, the 20 amino acid compounds listed in table 3 (0.5mol/l) with benzophenone (**2**) (0.5 mol/l) in pyridine/H_2O, 4:1, were processed (20 h, T = 37°C). Solvents were evaporated in vacuum and the crude reaction mixtures were analyzed by ^{1}H NMR spectroscopy. The percentages of converted amino acids were established by comparing the integration of the educt′s methyl ester singlet to that of the sum of new singlets appearing at δ = 4.0–3.3. In this way, all products were accounted for that conserved a methyl ester function, i.e. coupling products and amino acid dimers, but the γ-lactones were not detected. This might become a problem as almost quantitative cyclization to the γ-lactone **16** occured with AcPheOMe in pyridine/H_2O. Favourable signal separation allowed in the cases of AcHisOMe,·HOAc, and AcTrpOMe to integrate acetyl signals and thus to include possible γ-lactones. The differences with the amino acid consumption calculated on the basis of methyl ester signals were below 2%. This is the margin of error for the calculated conversions.

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