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Synthesis of Photoactive α-Mannosides and Mannosyl Peptides and Their Evaluation for Lectin Labeling

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Adhesion to the glycosylated surface of eukaryotic cells, mediated by lectins for example, plays an important role in inflammation and other cellular processes of living organisms. To elucidate the mechanisms involved in the adhesion to cell surfaces and their biological consequences, the investigation of the molecular interactions between carbohydrate recognition domains of lectins and their ligands is of relevance. In this work, we have selected the photoaffinity labeling technique for the exploration of the ligand binding to mannose-specific lectins, particularly the α-mannose-specific adhesin FimH, which is expressed at the tips of type 1 fimbriae of *Escherichia coli* bacteria. We have designed and synthesized a series of mannosides and glycopeptides derived thereof that are equipped with a photoactive functional group. It was our goal to compare the properties and labeling potencies of different types of photolabile residues, and therefore, photolabeled mannosides with an azide, a diazirine, and a benzophenone moiety were synthesized. Their crosslinking activity was investigated by photolysis in the presence of six different amino acids and with three model peptides, angiotensin II, PTHIKWGD, and pentaglycine as well. The crosslinked adducts so obtained were analyzed by mass spectrometry. In addition, difunctionalized mannosides were sought that contained a photolabel and a biotin marker to facilitate the isolation and the eventual identification, respectively, of the photolabeled peptides and proteins. To realize this concept, we have employed orthogonally functionalized glycoamino acid building blocks, which could be utilized as scaffold molecules for the synthesis of our bifunctional target molecules.

Introduction

Photoaffinity labeling is a straightforward technique used to explore molecular interactions of biomolecules such as ligand binding to carbohydrate-specific proteins. Introduced over 40 years ago,[1] the general principle of the photoaffinity methodology still holds. Upon irradiation, the photoprobe – a ligand that carries a photolabile moiety – is converted into a highly reactive intermediate, which is able to form a covalent bond with its receptor by insertion into C–H, N–H, or O–H bonds.[2] The resulting adduct can be analyzed by mass spectrometry after enzymatic digestion of the labeled protein.

It is our goal to utilize photoaffinity-labeled carbohydrates to assist in the investigation of the biological processes that occur on cell surfaces, such as cell–cell communication and bacterial adhesion. Involved in most of these adhesion processes are carbohydrate-binding proteins called lectins and selectins.[3] The interaction of lectins with the cell surface carbohydrates influences cellular events such as cell division or immune response.[4] Bacteria utilize their own lectins, which are assembled as part of the bacterial appendages, named fimbriae or pili,[5] for adhesion and colonization of host cell surfaces. Uropathogenic *Escherichia coli* express so-called type 1 fimbriae, which have been shown to play a crucial role in adhesion, infection, invasion, and pathogenicity.[6] The lectin domain of type 1 fimbriae is represented by a protein called FimH,[7] which is specific for α-mannosyl residues. The molecular details of carbohydrate binding to FimH are not yet fully understood. X-ray studies have revealed that FimH has a carbohydrate recognition domain (CRD) at its tip, which is compatible with mannose binding;[8] however, binding experiments with various multivalent mannosyl clusters[9] as well as theoretical studies[10] have suggested that there might be additional carbohydrate binding domains on FimH.

We have envisaged photoaffinity labeling of FimH with suitable α-mannoside derivatives equipped with a suitable photoreactive functional group (PAFG) to further investigate the interactions of FimH with carbohydrates.[11] The photoaffinity labeling technique has experienced considerable development and improvement during the last years, and various optimized photolabile residues, such as aryl azides,[12] diazirines,[13] and benzophenones,[14] have been successfully used. Since it can hardly be predicted which of these photoactive groups is most suited for photoaffinity labeling in a particular case,[15] it became our goal to synthesize and investigate a collection of α-mannosides that carry different photolabels and compare the resulting pho-

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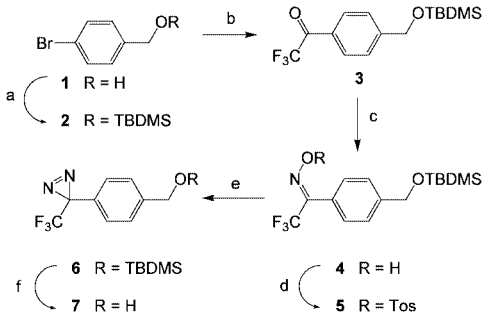
toactive mannosides in a series of irradiation experiments to elucidate their photochemical properties and labeling potencies. To the best of our knowledge, this is the first study on photolabeling of simple protected amino acids (threonine, serine, tyrosine, isoleucine, lysine, and arginine) on the one hand and model peptides (angiotensin II, PTHIKWGD and pentaglycine) on the other with the employment of various photophores in different ratios with the respective target molecule.

Results and Discussion

As photolabels (PALs), a diazirine ring, an aryl azide moiety, and a benzophenone substructure were envisaged, which would generate carbenes, nitrenes and radicals, respectively, upon photolysis.[12–14] On the basis of molecular modeling studies[16] and with the use of the published 3D structure of our target protein, FimH,[8] we decided to introduce these photolabels in the aglycon part of α-mannoside ligands. Furthermore, we synthesized mannosyl peptides as bifunctional scaffold molecules that allow for both photolabeling and biotinylation. Finally, investigation of synthesized molecules **12**, **14**, **16**, and **19** in different photolysis experiments is described here.

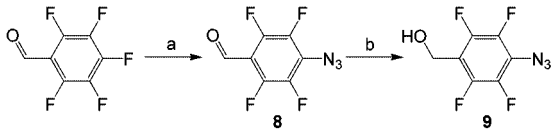
Synthesis of Hydroxy-Functionalized Photolabels 7 and 9

For the synthesis of photolabeled mannose derivatives, hydroxy-functionalized photoactive groups were needed in order to allow for the introduction of a mannosyl residue by glycosylation of the hydroxy group. We selected a diazirine ring as the first photoactive group to be introduced into our target molecules. We sought a diazirine substructure lacking α-hydrogens in order to avoid hydrogen abstraction after irradiation. This is a notorious problem when diazirine derivatives with α-hydrogens are used for photoaffinity labeling.[13d,17] The carbenes, which are delivered after irradiation of diazirines with UV light, often do not insert into C–H, N–H, or O–H bonds of the receptors under investigation. Instead, the carbenes undergo intramolecular hydrogen abstraction, which leads to unreactive alkenes. To circumvent this undesired side reaction, as well as to suppress the isomerization to the diazo analog, we selected 3-trifluoromethyl-3-phenyl diazirine[13,18] as the photolabel, which had to be functionalized in order to allow for the subsequent introduction of a mannosyl residue. Thus, *p*-bromobenzyl alcohol (**1**) was selected as the starting material, and it was protected as a silyl ether according to the literature[13] to form **2** (Scheme 1). It was then converted to trifluoroacetate **3** with freshly prepared *N*-trifluoroacetyl piperidine after dehalogenation with butyllithium.[19] Ketone **3** was used as the starting material for the synthesis of the desired diazirine. This is normally obtained after the conversion of a ketone into a diaziridine intermediate[13b,19,20] followed by oxidation. However, in the case of ketone **3**, the corresponding diaziridine did not form possibly because of the stereoelectronic effects of the aromatic ring system. Therefore, **3** was first activated with hydroxylamine hydrochloride to yield corresponding oxime **4**, and subsequently reacted with *p*-toluenesulfonyl chloride to give tosylated oxime **5**, which is equipped with a highly efficient leaving group. Tosyloxime **5** was then reacted with liquid ammonia overnight in dry ethyl ether at –60 °C to form the unstable diaziridine, which was not isolated as reported[13a–c] but oxidized in situ with iodine in MeOH to afford *tert*-butyldimethylsilyl-protected diazirine **6** in good yield. Removal of the TBDMS-protecting group with 10% hydrochloric acid in dry MeOH gave the desired hydroxy-functionalized [*p*-(hydroxymethyl)phenyl](trifluoromethyl)diazirine **7**. Analytical data for **7** were in accordance with the literature; its purification was possible by flash chromatography.



Scheme 1. a) TBDMS-Cl, imidazole, dry DMF, room temp., 87%; b) *n*BuLi, dry THF, argon, –30 °C, *N*-trifluoroacetyl piperidine, –50 °C, argon, 89%; c) hydroxylamine hydrochloride, dry EtOH, argon, 60 °C, 60%; d) tosyl chloride, *N,N*-DMAP, triethylamine, dry CH_2Cl_2, argon, 0 °C, room temp., 65%; e) NH_3, dry diethyl ether, –60 °C, triethylamine, 10% I_2 in MeOH, 0 °C → room temp., 55%; f) 10% HCl in dry MeOH, room temp., 77%.

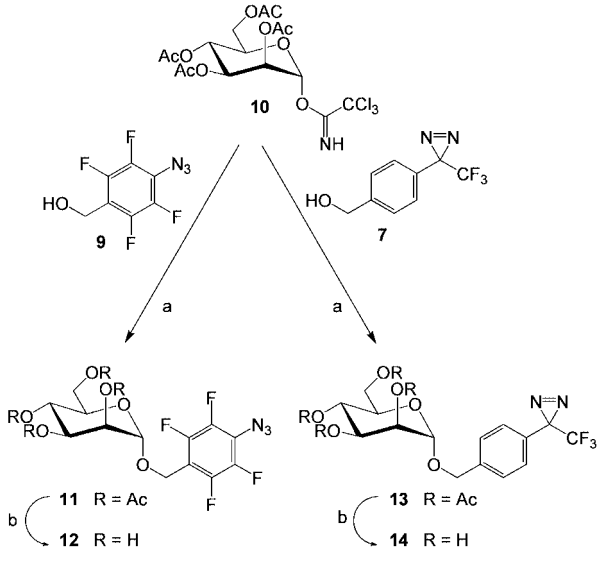
Another photoactive group we aspired to incorporate into the mannosides is the aryl azide moiety, which is among the most common photoactive reagents.[12,13] However, it has been reported that phenyl azides show almost no insertion products after irradiation in hydrocarbon solvents at room temperature.[21] Instead, the singlet nitrenes that are generated by photolysis undergo rapid ring expansion to form dehydroazepines[22] that are either intercepted by nucleophiles to give amines, or in the absence of nucleophiles undergo polymerization.[23] When polyfluorinated aryl azides such as pentafluorophenyl azides are used as photoactive reagents, irradiation leads to a stabilized singlet nitrene, which is less prone to undesired ring expansion, and is more readily trapped by nucleophiles, such as amines or alcohols and even inactivated alkanes, to form stable covalent adducts.[21,24] Thus, we synthesized an aryl azide from pentafluorobenzaldehyde, which was reacted with sodium azide in acetone–water to form *p*-azidobenzaldehyde **8** after regioselective nucleophilic aromatic substitution in an improved yield as compared to the literature[21] (Scheme 2). Chemoselective reduction of the aldehyde group in **8** was then accomplished with the dimethylamine–borane complex in acetic acid[25] to provide the literature-known benzyl alcohol **9**[21] in a yield of 89% after chromatographic purification.



Scheme 2. a) NaN_3, acetone–water (1:1), reflux (100 °C), 95%; b) dimethylamine–borane complex, 98% AcOH, 55 °C, 89%.

Synthesis of Photolabeled Mannosides 12 and 14

With the employment of photolabels **7** and **9**, mannosides with a photoactive aglycon moiety could be obtained by glycosylation with the use of acetyl-protected mannosyl trichloroacetimidate **10** under Lewis acid catalysis.[26] Azido-functionalized benzyl alcohol **9** was mannosylated with TMSOTf to yield acetyl-protected mannoside **11** in 69% yield after column chromatography. This was subsequently deprotected according to Zemplén[27] with freshly prepared sodium methoxide in methanol (Scheme 3). After purification by flash chromatography on reverse-phase (RP) silica, desired photolabile α-mannoside **12**, which carries the azide functionality, was obtained in a yield of 89%. The analogous procedure that starts with diazirine **7** led to acetylated α-mannoside **13** in 69% after purification. Removal of the acetyl protecting groups of **13** was successful with Zemplén reaction conditions and freshly prepared sodium methoxide reagent. After RP-flash chromatography, desired photolabile α-mannoside **14** equipped with the diazirine moiety was obtained in a yield of 88%.



Scheme 3. a) 0.3 equiv. TMSOTf, dry CH_2Cl_2, argon, room temp., 69% for **11** (56 h), 69% for **13** (5 d); b) 1 M NaOMe in dry MeOH, argon, room temp., 89% for **12**, 88% for **14**.

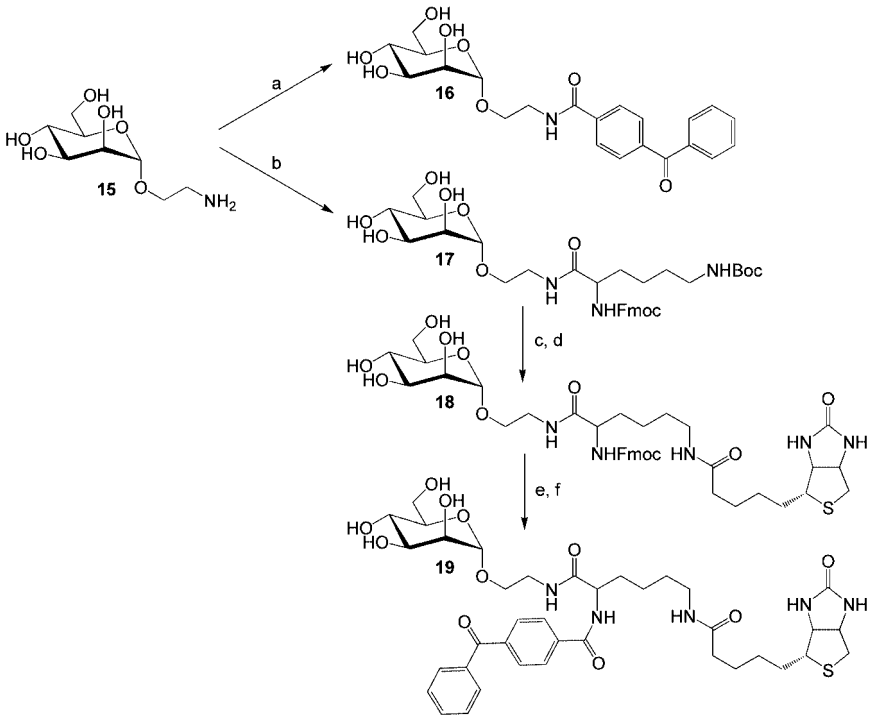
The mannosylation reactions which afford **11** and **13** required modified reaction conditions because of the lower reactivity of corresponding benzyl alcohols **7** and **9**. The reactivity of **7** turned out to be even lower than that of **9**. To obtain complete conversion of both acceptor alcohols **7** and **9**, reaction temperature, reaction time, and the amount of catalyst had to be increased.

Mannosides **12** and **14** showed similar potency as inhibitors of type 1 fimbriae-mediated bacterial adhesion as the standard inhibitor of this carbohydrate-protein interaction *p*-nitrophenyl α-D-mannoside.[28] Thus, they can be regarded as suitable candidates for the photoaffinity labeling of FimH. Next, we wanted to explore a benzophenone substructure as the third photolabel in our study as it has been reported that benzophenones are also advantageous photoactive groups.[14a] Photolabile benzophenones have been employed as photophores either to functionalize remote C–H bonds in biomolecules or to map binding sites and conformational properties of nucleotides or proteins.[14a] They have three major chemical and biochemical advantages; first, they show higher stability than arylazides and diazirines; second, benzophenones can be used under ambient light without any decomposition, and protein damage is avoided because photolysis is achieved at wavelengths of 350-360 nm, third, the benzophenone group reacts preferentially with unreactive C–H bonds in hydrophobic parts of the biomolecules even in the presence of water or bulky nucleophiles.

To introduce the benzophenone photolabel, we envisaged the peptide coupling of 4-benzoylbenzoic acid with amino-functionalized mannoside **15**,[29] which is easily obtained from peracetylated mannose in three steps.[30] This turned out to be a most convenient synthetic route which led to photoactive mannoside **16** in a HATU-mediated peptide coupling reaction[31] (Scheme 4). The pure product was obtained after purification on silica gel followed by RP-HPLC in a yield of 52%.

Our next consideration was dedicated to the isolation and analysis of the photoaffinity-labeled adducts. Photolysis of a photoprobe–protein mixture followed by proteolytic digestion of the photolabeled protein can lead to a complex mixture of nonlabeled fragments together with the desired photocrosslinked adduct as well as a number of undesired side products. MS analysis of such a mixture is complicated because of the small mass differences of the receptor fragments in the obtained mixture. To facilitate the detection as well as the isolation of the photocrosslinked products, we envisaged a strategy that would allow for the introduction of a biotin tag into our photoprobes.[32] An incorporated biotin tag would allow for both the determination of the concentration of the insertion products by avidin-biotin affinity chromatography and the detection of the products through the combination of SDS gel electrophoresis and Western blotting technique.[33–35]

A bifunctional mannosyl peptide was selected as the scaffold for the synthesis of a biotinylated photoprobe. Aminoethyl mannoside **15** was coupled with an orthogonally protected lysine derivative in a HATU-mediated reaction to afford bifunctional glycopeptide **17**, which carries two orthogonally protected amino groups in the aglycon moiety (Scheme 4). Diamine **17** formed the starting material for the introduction of the photoactive group as well as the biotin tag. In situ removal of the *N*-*tert*-butyloxycarbonyl protecting group followed by its peptide coupling with (+)-biotin led to tagged glycopeptide **18** in 69% yield after purification



Scheme 4. a) 4-Benzoylbenzoic acid, HATU, DIPEA, dry DMF, argon, room temp., 52%; b) Boc-Lys-Fmoc-OH, HATU, DIPEA, dry DMF, argon, room temp., 87% (crude); c) 80% TFA, room temp.; d) (+)-biotin, HATU, DIPEA, dry DMF, argon, room temp., 69% (two steps); e) 20% piperidine, DMF; f) 4-benzoylbenzoic acid, HATU, DIPEA, dry DMF, argon, room temp., 40% from **15**.

on silica gel. The α-amino function of lysine was then deprotected with 20% piperidine in DMF and the subsequent introduction of 4-benzoylbenzoic acid by standard peptide coupling techniques in DMF led to the desired bifunctional photoactive mannoside. Purification was accomplished by two consecutive flash chromatographic separations on silica gel to provide pure **19** in 40% overall yield from **15**.

Irradiation Experiments

Photoaffinity labeling photophores are incubated with a target peptide or protein in the dark and then irradiated to be crosslinked with their receptors. The resulting covalent adduct can be analyzed with MS techniques such as MALDI-TOF. Synthesized photolabeled mannosides **12**, **14**, and **16** and **19** should form highly reactive intermediates such as nitrenes, carbenes, and radical ions, respectively, upon irradiation with UV light. These are able to insert into C–H, N–H or O–H bonds of biomacromolecules, the protein FimH for instance. To check this ability we performed a series of different photolysis experiments.

For all PALs, absorption maxima (λ_{max}) were determined, and upon irradiation a decrease in λ_{max} was observed with time. Therefore, stock solutions of photoprobes **12**, **14**, **16**, and **19** were prepared in doubly distilled water or MeOH. Because of its poor solubility in water, arylazide **12** had to be dissolved in MeOH. To monitor the decrease in λ_{max}, each sample was irradiated with UV light ≥320 nm up to 60 min and UV spectra were recorded in 5 min intervals to obtain information about the irradiation time required to transfer the photolabile residues into their respective reactive intermediates (Table 1). All tested photolabile compounds exhibit absorption maxima at wavelengths >320 nm; thus, they all qualify as photoprobes for the investigation of biomacromolecules by photoaffinity labeling without decomposition of the target receptors.

Table 1. UV spectroscopic data for the synthesized photolabels **12**, **14**, **16**, and **19**.

Compound (PAL)	Solvent	λ_{max} [nm]	Disappearance of λ_{max} [min][a]
12 (azide)	MeOH[b]	343.3	30
14 (diazirine)	doubly distilled H_2O	355.5	10
16 (benzophenone)	doubly distilled H_2O	330.0	[c]
19 (benzophenone)	doubly distilled H_2O	327.8	[c]

[a] After irradiation at wavelengths ≥320 nm; upon irradiation, UV spectra were recorded in 5 min intervals. [b] Azide **12** is only poorly soluble in water. [c] No decrease in λ_{max} because of the relaxation of the excited triplet radical to the ground state.

MS analyses of the irradiated solutions showed the formation of insertion products with the solvent in case of **12** and **14**. Benzophenone-labeled derivatives **16** and **19** did not show insertion products of any sort, which indicates their lower reactivity. This is due to the relaxation of the excited triplet diradical that is initially formed during irradiation.[14a]

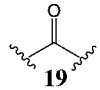
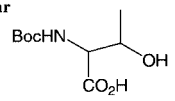
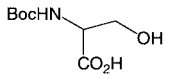
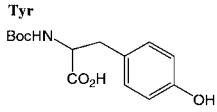
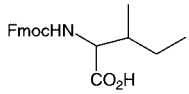
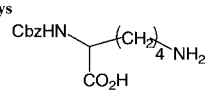
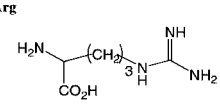
A number of amino acids were then selected for photolabeling with PALs **12**, **14**, **16**, and **19**. Table 2 summarizes the details of the labeling experiments with six protected

amino acid derivatives derived from threonine, serine, tyrosine, isoleucine, lysine, and arginine. Irradiation of photoprobes **14**, **16**, and **19** with all amino acids was carried out in doubly distilled water, whereas experiments with azide **12** as the photophore required MeOH as the solvent. The concentration of each PAL solution was chosen as 0.1 mM and irradiation experiments were carried out with four different amino acid concentrations (0.05 mM, 0.2 mM, 0.5 mM, and 1.0 mM) for each amino acid.

MS analyses of the irradiated PAL-amino acid samples revealed if an insertion product had been formed, as well as the mass of the formed insertion product. However, neither the chemoselectivity nor the regioselectivity of the photolabeling reaction could be deduced from the MS data. For example, it cannot be determined whether the carbene derived from diazirine **14** inserted into the O–H bond or a C–H single bond of threonine. According to the literature,[11,12] carbenes tend to insert into polar functionalities such as hydroxy groups or amino groups rather than into nonpolar C–H bonds. On the other hand, benzophenone can be assumed to produce C–H insertion products upon irradiation. This assumption is supported by the fact that **16** and **19** do not form insertion products with water (Table 1) or polar amino acids such as threonine or serine (Table 2). Furthermore, all investigated PALs seem to be able to form insertion products, as irradiation with certain side-chain-functionalized amino acids led to crosslinked products that were detected by mass spectrometric methods.

We found that insertion products of all the PALs investigated were formed with one of the amino acids, whereas their exact structure remained unknown. However, at this point we were not interested in the structural analysis of the formed insertion products, but instead we wanted to know if our photoactive mannosides, which were successful with amino acids would also work with peptides. Therefore, two octapeptides and pentaglycine were selected as model peptides and irradiated. Angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) and PTHIKWGD (H-Pro-Thr-His-Ile-Lys-Trp-Gly-Asp-OH) were chosen to reflect a broad variety of amino acid side chains, whereas pentaglycine provided only unreactive C–H-bonds for insertion. Sample mixtures containing 10 equiv. of peptide (1.0 mM)

Table 2. Results of the photolabeling experiments of various protected amino acids with PALs **12**, **14**, **16**, and **19** in solution as detected by mass spectrometry.

	 **12** M = 383.07	 **14** M = 378.30	 **16** M = 431.43	 **19** M = 785.90
Thr  M = 219.23	no insertion	insertion product *m/z* = 568.453	no insertion	no insertion
Ser  M = 205.21	no insertion	no insertion	no insertion	no insertion
Tyr  M = 281.30	no insertion[a]	no insertion[a]	no insertion[a]	no insertion[a]
Ile  M = 339.39	insertion product *m/z* = 707.309	no insertion	insertion product *m/z* = 763.353[b]	no insertion
Lys  M = 280.32	no insertion	insertion product *m/z* = 631.418	*m/z* = 710.849	insertion product *m/z* = 1066.289[c]
Arg  M = 174.20	no insertion	insertion product *m/z* = 523.341	no insertion	no insertion

[a] Limited solubility of amino acid component. [b] Decarboxylation of the amino acid was observed. [c] Atmospheric oxidation of the biotin sulfur during photolysis formed the respective biotin sulfone.

Table 3. Monoisotopic masses of the insertion products formed from the reaction of PALs with three different model peptides.

Peptide	Crosslinked products with **12**	Crosslinked products with **14**	Crosslinked products with **16**	Crosslinked products with **19**
Angiotensin II (M = 1046.54)	m/z = 1415.490[a]	m/z = 1396.641[a]	no insertion	m/z = 1848.192[b]
PTHIKWGD (M = 953.07)	m/z = 1308.689	m/z = 1326.588	no insertion	no insertion
Pentaglycine (M = 303.27)	–	–	m/z = 733.848	no insertion

[a] Further analysis by fragmentation with the use of ESI-FT-ICR-MS–MS. [b] Atmospheric oxidation of the biotin sulfur atom during photolysis formed the respective biotin sulfone.

and one equiv. of photolabel (0.1 mM) in doubly distilled water were prepared and irradiated with UV light ≥320 nm for 10 min (diazirine **14**), 30 min (azide **12**), and 60 min (benzophenones **16** and **19**). Formed insertion products were concentrated by "ZipTipping"®[36] and analyzed with MALDI-TOF-MS and ESI-MS. MS analyses of the irradiation experiments with those model peptides showed crosslinked products in the case of angiotensin II with **12**, **14**, and **19**, and in case of PTHIKWGD with **12** and **14**. Photoprobe **16** showed no reaction with these two peptides but was crosslinked with pentaglycine (Table 3).

Diazirine **14** and azide **12** were fragmented in an ESI-FT-ICR-MS–MS experiment to further characterize the crosslinked products formed upon their reaction with irradiated angiotensin II. In particular, we were interested to learn if different photoprobes attack different positions of the investigated model peptides, and to determine whether they show preferences for certain functional groups. MS–MS analysis of **12**-angiotensin II, the insertion product resulting from the irradiation of azide **12** with the peptide, and **14**-angiotensin II, the insertion product resulting from the irradiation of diazirine **14** and the peptide, provided fragmentation patterns that are compatible with the corresponding data found in the ProteinProspector Database. However, it was obvious that the covalent linkage between the respective PAL and the peptide was cleaved before fragmentation of the peptide backbone and consequently, no information about the photolabeling position could be obtained from this analysis. We are currently developing this technology further to eventually allow for the accurate determination of labeling sites in biomolecules.

As the irradiation of model peptides with **12**, **14**, and **16** delivered a high amount of unlabeled peptide and only a low yield of crosslinked adduct, a method to concentrate and purify insertion products was required to facilitate the analytical potential of this technology. Therefore, mannoside **19** containing the benzophenone photolabel and a biotin tag was employed for the photoaffinity labeling experiment. After its irradiation with angiotensin II, a biotin-tagged insertion product could be isolated and concentrated with a combination of cationic and affinity chromatography and an avidin cartridge facilitated MALDI-TOF-MS analysis of the concentrated crosslinked adduct. Again, mass spectrometric analysis indicated the oxidation of the biotin sulfur atom to form the respective biotin sulfone.

Conclusions

With the aim to investigate carbohydrate binding to the mannose-specific lectin FimH, we have synthesized mannosides **12**, **14**, and **16** equipped with three different photoactive functional groups: a diazirine, an azide, and a benzophenone moiety, respectively. Furthermore, to assist the photoaffinity technique by biolabeling, we have synthesized biotin derivative **19**. We then compared the potential of these three photophores in photoaffinity labeling.

All of the synthesized photoprobes exhibit absorption maxima at wavelengths >320 nm and can therefore be used in the investigation of biomacromolecules such as peptides and proteins without degradation. During their photolysis in water or MeOH, photophores **12** and **14** formed covalent insertion products. Further studies that include illumination experiments in the presence of six different amino acids as well as the three model peptides, angiotensin II, PTHIKWGD, and pentaglycine, allowed further conclusions about the features of the investigated PALs. To obtain the highest amount of photocrosslinked product it was determined that the optimal ratio of photolabel to target molecule is 1:10. Although each of the synthesized PALs is able to crosslink to biomolecules, the exact position of the formed covalent bond could not be elucidated unequivocally.

According to our irradiation experiments, we found that the diazirine group of PAL **14** seemed to be the most suitable photoprobe for the photolabeling of peptides. The short irradiation time, an absorption maximum >320 nm, its good solubility in water, and its preference for the insertion into polar groups such as amines and OH groups makes the diazirine moiety an advantageous photolabel for studies in biological chemistry. Benzophenones on the other hand showed some disadvantages, such as steric hindrance, increased irradiation times, and limited reactivity. However, its low reactivity, which is due to the relaxation of the excited state to the ground state, also limits undesired side reactions with the solvent for instance. Moreover, the hydrophobic benzophenone photolabel preferentially inserts into apolar C–H bonds.

Tetrafluorinated aryl azide **12** is less suited for photoaffinity labeling. Because of its poor solubility in water, it is less useful for the investigation of larger peptides or proteins under physiological conditions. An increased tendency

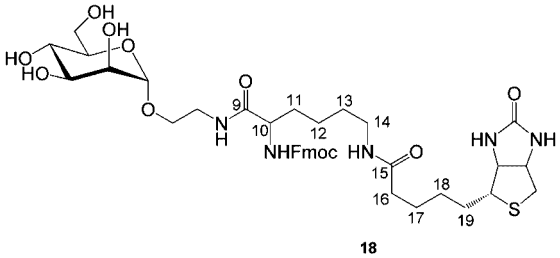
to unspecific side reactions, which complicate the analysis of the irradiation products combined with very long irradiation times are central disadvantages of the azide photolabel.

These results form the basis for our future work, which will be directed towards the optimization of the photoaffinity methodology, and the determination of the crosslinking sites in lectin CRDs. As part of this endeavor, we have synthesized biotin-labeled photoactive mannoside **19**, which has facilitated the isolation and identification of photocrosslinked peptides.

Experimental Section

General Remarks: Optical rotations were measured with a Perkin–Elmer polarimeter (22 °C, 589 nm, length of cell = 1 dm). Reactions were monitored by TLC on silica gel GF_{254} (Merck) with detection under UV light and by charring with 10% sulfuric acid in ethanol or with anisaldehyde and subsequent heating. Flash column chromatography was performed on silica gel 60 (40-63 μm, Merck) and for RP-MPLC a Merck Licroprep RP-18 column (Büchi) was used. Preparative HPLC was performed with a Shimadzu LC-8a (LiChrosorb RP-8, HIBAR). NMR spectra were recorded with Bruker AMX 400, Bruker DRX 500, or Bruker Avance 600 instruments. Chemical shifts are relative to TMS or the solvent peaks of $CDCl_3$ (δ =7.24 ppm for ^{1}H NMR, 77.0 ppm for ^{13}C NMR) or MeOD (δ =3.35 ppm and 4.78 ppm for ^{1}H NMR, 49.3 ppm for ^{13}C NMR). Where necessary, assignments were based on 2D experiments (COSY, HSQC, HMBC, or NOESY). IR spectra were taken with a Perkin–Elmer FT IR Paragon 1000. MALDI-TOF mass spectra were measured with a Bruker Biflex III with a 19 kV acceleration voltage. 4-Hydroxy-α-cyanocinnamic acid (HCCA) was used as the matrix, either as a saturated solution in a solvent mixture (33% MeCN/doubly distilled water and 0.1% TFA) or as a saturated solution in acetone. Ionization was effected with a nitrogen laser at 337 nm. ESI-MS spectra were measured with an Applied Biosystems Mariner ESI-TOF 5280. ESI-FT-ICR-MS/MS spectra were measured with a Bruker 7 Tesla Apex II mass spectrometer at the Research Center Borstel. Fragmentation patterns and sequence analysis were determined with ProteinProspector as the protein sequence database. Millipore C_{18}-pipette tips were used for ZipTipping® and for cationic and affinity chromatography ICAT®-kits from Applied Biosystems were used.

For assignment of the NMR spectroscopic data **18** and **19** were numbered according to the following drawing:



18

When helpful, the mannosyl substructure is abbreviated as "man" and in complicated cases lengthy IUPAC names were substituted by simplified terms.

***p*-Azidotetrafluorobenzyl-2,3,4,6-tetra-*O*-acetyl α-D-Mannopyranoside (11):** Acetyl-protected mannosyl trichloroacetimidate **10** (1.70 g, 3.50 mmol) was dissolved in dry DCM (20 mL) under an argon atmosphere. *p*-Azidotetrafluorobenzyl alcohol (**9**, 0.83 g, 3.90 mmol) was then added under argon, followed by the dropwise addition of a freshly prepared TMSOTf solution (0.3 M *in* dry DCM, 3.3 mL) at room temp. The resulting reaction mixture was kept under an argon atmosphere and stirred for 56 h in the dark. Evaporation of the solvent and purification of the crude product by flash chromatography (toluene/ethyl acetate, 2:1) delivered acetyl-protected mannoside **11** as a pure solid (1.32 g, 2.40 mmol, 69%). R_f = 0.55 (toluene/ethyl acetate, 2:1). FTIR (KBr): $\tilde{\nu}$ = 2975.7, 2124.7, 1739.9, 1654.5 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ = 5.25 (dd, J = 9.9, 3.0 Hz, 1 H, 4-H), 5.23 (dd, J = 3.7, 3.2 Hz, 1 H, 3-H), 5.15 (dd, J = 3.6, 1.7 Hz, 1 H, 2-H), 4.85 (d, J = 1.7 Hz, 1 H, 1-H), 4.75 (dt, J = 11.0, 1.8 Hz, 1 H, benzyl-C*H*H,), 4.54 (dt, J = 11.0, 1.6 Hz, 1 H, benzyl-CH*H*), 4.23 (dd, J = 12.3, 5.4 Hz, 1 H, 6a-H), 4.03 (dd, J = 12.3, 2.4 Hz, 1 H, 6b-H), 3.92 (ddd, J = 9.9, 5.5, 2.4 Hz, 1 H, 5-H,), 2.15, 2.07, 1.98, 1.92 (4 s, 12 H, 4 CH_3, 4 OAc) ppm. ^{13}C NMR (125.75 MHz, MeOD, 25 °C): δ = 170.62, 169.96, 169.82, 169.72 (4 C=O, 4 OAc), 146.60 (d, $J_{C,F}$ = 156.2 Hz, aryl-C-F), 144.62 (d, $J_{C,F}$ = 164.2 Hz, aryl-C-F), 141.41 (d, $J_{C,F}$ = 106.7 Hz, aryl-C-F), 139.42 (d, $J_{C,F}$ = 115.4 Hz, aryl-C-F), 120.96 (t, $J_{C,F}$ = 84.3 Hz, aryl-C-N_3), 110.07 (t, $J_{C,F}$ = 126.1 Hz, aryl-C-CH_2,), 97.39 (C-1), 69.31 (C-2), 69.09 (C-5), 68.78 (C-3), 65.95 (C-4), 62.35 (C-6), 56.61 (benzyl-C), 20.82, 20.65, 20.60, 20.42 (4 CH_3, 4 OAc) ppm. MALDI-TOF-MS: m/z = 574.3 [M + Na]$^+$, 590.3 [M + K]$^+$. ESI-HRMS: calcd. for $C_{21}H_{21}F_4N_3NaO_{10}$ [M + Na]$^+$ 574.1055; found 574.1039.

***p*-Azidotetrafluorobenzyl α-D-Mannopyranoside (12):** Mannoside **11** (600 mg, 1.10 mmol) was dissolved in dry methanol (12 mL), and a freshly prepared mixture of sodium methoxide in dry methanol (1 M, 0.5 mL) was carefully added. The resulting mixture was stirred at room temp. for 4 h, and then acidic ion exchange resin (Amberlite IR-120) was added in portions until pH 6 was reached. The mixture was filtered, and the filtrate evaporated in vacuo. Purification of the crude product by flash chromatography with RP-silica gel (ethyl acetate/methanol/water, 6:2:1) delivered the title compound (371 mg, 0.97 mmol, 89%) as light orange crystals. R_f = 0.66 (ethyl acetate/methanol/water, 6:2:1). M.p. 159 °C. $[\alpha]_D$ = + 36.5 (c = 2.0, MeOH). UV: $\lambda_{max}(1)$ = 343.3 nm (c = 0.10 mM, MeOH), $\varepsilon(1)$ = 1500 L mol^{-1} cm^{-1}, $\lambda_{max}(2)$ = 250.6 nm (c = 0.10 mM, MeOH), $\varepsilon(1)$ = 16300 L mol^{-1} cm^{-1}. FTIR (KBr): $\tilde{\nu}$ = 3464.6, 3417.4, 3370.2, 3275.6, 2925.0, 2896.2, 2120.0, 1489.7, 1240.8, 1067.2 cm^{-1}. ^{1}H NMR (500 MHz, MeOD, 25 °C): δ = 4.92 (d, J = 1.6 Hz, 1 H, 1-H), 4.89 (dt, J = 11.6, 1.8 Hz, 1 H, benzyl-C*H*H), 4.70 (dt, J = 11.6, 1.7 Hz, 1 H, benzyl-CH*H*), 3.82 (dd, J = 11.8, 2.4 Hz, 1 H, 6a-H), 3.81 (dd, J = 3.0, 1.7 Hz, 1 H, 2-H), 3.77 (dd, J = 11.8, 5.5 Hz, 1 H, 6b-H), 3.67 (dd, J = 9.8, 2.7 Hz, 1 H, 4-H), 3.66 (dd, J = 2.9, 2.6 Hz, 1 H, 3-H), 3.56 (ddd, J = 9.8, 5.5, 2.3 Hz, 1 H, 5-H) ppm. ^{13}C NMR (125.75 MHz, MeOD, 25 °C): δ = 147.90 (d, $J_{C,F}$ = 179.5 Hz, aryl-C-F), 145.93 (d, $J_{C,F}$ = 212.0 Hz, aryl-C-F), 142.86 (d, $J_{C,F}$ = 179.0 Hz, aryl-C-F), 140.89 (d, $J_{C,F}$ = 227.5 Hz, aryl-C-F), 121.78 (t, $J_{C,F}$ = 222.7 Hz, aryl-C-N_3), 112.91 (t, $J_{C,F}$ = 130.0 Hz, aryl-*C*-CH_2), 101.96 (C-1), 75.09 (C-5), 72.04 (C-4), 71.99 (C-2), 68.40 (C-3), 62.79 (benzyl-*C*H_2), 57.50 (C-6) ppm. MALDI-TOF-MS: m/z = 406.3 [M + Na]$^+$, 421.3 [M + K]$^+$. ESI-MS: m/z = 406.06 [M + Na]$^+$, 789.13 [2M + Na]$^+$. ESI-HRMS: calcd. for $C_{13}H_{13}F_4N_3NaO_6$ [M + Na]$^+$ 406.0633; found 406.0678. $C_{13}H_{13}F_4N_3O_6$ (383.25): calcd. C 40.72, H 3.42, N 10.97; found C 39.78, H 3.02, N 11.37.

3-Trifluoromethyl-3-[*p*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyloxymethyl)phenyl]diazirine (13): Acetyl-protected mannosyl trichloroacetimidate **10** (0.62 g, 1.26 mmol) was dried in vacuo for 20 min prior to use and dissolved in dry DCM (15 mL) under an argon atmosphere. Diazirine derivative **7** (0.32 g, 1.50 mmol)

was then added, followed by the dropwise addition of a freshly prepared TMSOTf solution (0.1 M in dry DCM, 4.5 mL) under a flow of argon. The reaction mixture was stirred in the dark at room temp. for 5 d. Evaporation of the solvent and purification of the crude product by flash chromatography (toluene/ethyl acetate, 2:1) delivered the title compound as a yellowish powder (566 mg, 1.04 mmol, 69%). R_f = 0.62 (toluene/ethyl acetate, 2:1). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.31 (dd, J = 8.6, 2.2 Hz, 2 H, H-aryl-H), 7.14 (dd, J = 8.0, 2.3 Hz, 2 H, aryl-H), 5.30 (dd, J = 9.9, 3.3 Hz, 1 H, 3′-H), 5.24 (dd, J = 9.7, 9.7 Hz, 1 H, 4′-H), 5.22 (dd, J = 3.2, 1.8 Hz, 1 H, 2′-H), 4.80 (d, J = 1.6 Hz, 1 H, 1′-H), 4.66 (d, J = 12.3 Hz, 1 H, benzyl-C*H*H), 4.50 (d, J = 12.3 Hz, 1 H, benzyl-CH*H*), 4.21 (dd, J = 12.3, 5.2 Hz, 1 H, 6′a-H), 4.00 (dd, J = 12.3, 2.5 Hz, 1 H, 6′b-H-), 3.91 (ddd, J = 9.5, 5.1, 2.5 Hz, 1 H, 5′-H), 2.13, 2.07, 1.89, 1.85 (4 CH_3, 4 OAc) ppm. ^{13}C NMR (75.45 MHz, $CDCl_3$, 25 °C): δ = 170.67, 170.07, 169.96, 169.75 (4 C=O, 4 OAc), 163.45 (aryl-C-N=N), 137.95 (aryl-*C*-CH_2), 129.05 (aryl-C), 128.30 (2 aryl-C), 126.74 (2 aryl-C), 122.20 (q, $J_{C,F}$ = 274.7 Hz, CF_3), 96.79 (C-1′), 77.22 (C-5′), 69.43 (C-3′), 69.02 (C-2′), 68.81 (benzyl-*C*H_2), 66.04 (C-4′), 62.41 (C-6′), 20.91, 20.78, 20.73, 20.72 (4 CH_3, 4 OAc) ppm. MALDI-TOF-MS: m/z = 519.4 [M – N_2 + H]$^+$. ESI-HRMS: calcd. for $C_{23}H_{25}F_3N_2NaO_{10}$ [M + Na]$^+$ 569.1525; found 569.1446.

3-Trifluoromethyl-3-[*p*-(α-D-mannopyranosyloxymethyl)phenyl]diazirine (14): Photolabile acetyl-protected mannoside **13** (297 mg, 0.50 mmol) was dissolved in dry methanol (14 mL) and a freshly prepared solution of sodium methoxide in dry methanol (2 mM, 1.1 mL) was added. The resulting mixture was stirred at room temp. for 1 h, acidic ion exchange resin (Amberlite IR-120) was then added in small portions until a pH 6.5 was reached. The mixture was filtered and the filtrate evaporated in vacuo. The product was purified by two consecutive flash column chromatography separations on RP-silica gel (ethyl acetate/methanol/water, 6:2:1) to yield pure photolabile mannoside **14** (166 mg, 0.44 mmol, 88%) as a light yellow powder. R_f = 0.68 (ethyl acetate/methanol/water, 6:2:1). $[\alpha]_D$ = + 44.5 (c = 1.9, H_2O). UV: $\lambda_{max}(1)$ = 355.5 nm (c = 0.1 mM, doubly distilled H_2O), $\varepsilon(1)$ = 1500 L mol^{-1} cm^{-1}, $\lambda_{max}(2)$ = 265.5 nm (c = 0.1 mM, doubly distilled H_2O), $\varepsilon(2)$ = 5200 L mol^{-1} cm^{-1}. FTIR (KBr): $\tilde{\nu}$ = 3431.8, 2929.3, 1663.7, 1342.0, 1154.1, 831.9 cm^{-1}. ^{1}H NMR (500 MHz, MeOD, 25 °C): δ = 7.52 (d, J = 8.5 Hz, 2 H, aryl-H), 7.28 (d, J = 8.1 Hz, 2 H, aryl-H), 4.87 (d, J = 1.7 Hz, 1 H, 1-H), 4.83 (d, J = 12.4 Hz, 1 H, benzyl-C*H*H), 4.61 (d, J = 12.4 Hz, 1 H, benzyl-CH*H*), 3.89 (dd, J = 3.4, 1.8 Hz,1 H, 2-H), 3.87 (dd, J = 11.8, 2.3 Hz, 1 H, 6b-H), 3.77 (dd, J = 9.4, 3.4 Hz, 1 H, 3-H), 3.75 (dd, J = 11.8, 5.9 Hz, 1 H, 6a-H), 3.67 (t, J = 9.5 Hz, 1 H, 4-H), 3.60 (ddd, J = 9.5, 6.0, 2.3 Hz, 1 H, 5-H) ppm. ^{13}C NMR (125.75 MHz, MeOD, 25 °C): δ = 166.26 (aryl-C-N=N), 141.54 (aryl-*C*-CH_2), 129.53 (2 aryl-C), 129.24 (aryl-C), 127.64 (2 aryl-C), 123.63 (q, $J_{C,F}$ = 273.8 Hz, CF_3), 100.94 (C-1), 75.03 (C-5), 72.60 (C-3), 72.11 (C-2), 69.02 (benzyl-*C*H_2), 68.61 (C-4), 62.89 (C-6) ppm. ESI-MS: m/z = 473.18 [M – N_2 + 2 MeOH]$^+$, 401.08 [M + Na]$^+$, 373.08 [M – N_2 + Na]$^+$. ESI-HRMS: calcd. for $C_{15}H_{17}F_3N_2NaO_6$ [M + Na]$^+$ 401.0931; found 401.0841.

Benzophenone Derivative 16: 2′-Aminoethyl α-D-mannoside **15** (200 mg, 0.90 mmol), HATU (330 mg, 0.90 mmol), and 4-benzoylbenzoic acid (225 mg, 1.00 mmol) were mixed in a Schlenk flask and dried in vacuo for 30 min. The solid mixture was then dissolved in dry DMF (12 mL) and DIPEA (0.34 mL, 2.00 mmol) was carefully added. The mixture turned yellow, and was stirred at room temp. for 17 h under an argon atmosphere. The solvent was then evaporated, and the resulting crude product was purified by flash chromatography on RP-silica gel (ethyl acetate/methanol/water, 6:2:1) followed by gradient RP-HPLC to obtain the product (201 mg, 52%) as a light yellow lyophilisate. R_f = 0.59 (ethyl acetate/methanol/water, 6:2:1). HPLC: retention time = 40.89 min, gradient = MeCN/H_2O, 38:62. $[\alpha]_D$ = + 29.0 (c = 2, H_2O). UV: $\lambda_{max}(1)$ = 330.0 nm (c = 0.25 mM, doubly distilled H_2O), $\varepsilon(1)$ = 266.7 L mol^{-1} cm^{-1}, $\lambda_{max}(2)$ = 258.1 nm (c = 0.25 mM, doubly distilled H_2O), $\varepsilon(2)$ = 14000 L mol^{-1} cm^{-1}. ^{1}H NMR (600 MHz, MeOD, 25 °C): δ = 8.00 (dd, J = 8.4, 1.8 Hz, 2 H, benzophenone-*m*-H), 7.87 (dd, J = 8.1, 1.8 Hz, 2 H, benzophenone-*o*-H), 7.83 (dd, J = 7.8, 1.2 Hz, 2 H, benzophenone-*o*-H), 7.71 (dd, J = 7.4, 1.2 Hz, 1 H, benzophenone-*p*-H), 7.59 (td, J = 7.6, 1.6 Hz, 2 H, benzophenone-*m*-H), 4.86 (d, J = 1.6 Hz, 1 H, 1-H), 3.93 (dd, J = 10.3, 4.8 Hz, 1 H, manOCH_2C*H*H), 3.87 (dd, J = 3.1, 1.7 Hz, 1 H, 2-H), 3.86 (dd, J = 12.0, 2.1 Hz, 1 H, 6a-H), 3.76 (dd, J = 6.2, 3.5 Hz, 1 H, 3-H), 3.75 (dd, J = 10.9, 4.6 Hz, 1 H, manOCH_2CH*H*), 3.73 (dd, J = 11.7, 5.8 Hz, 1 H, 6b-H), 3.71 (dd, J = 8.8, 7.3 Hz, 1 H, manOC*H*HCH_2), 3.66 (dd, J = 9.1, 7.3 Hz, 1 H, manOCH*H*CH_2), 3.65 (dd, J = 9.2, 6.9 Hz, 1 H, 4-H), 3.62 (ddd, J = 9.5, 5.9, 2.2 Hz, 1 H, 5-H) ppm. ^{13}C NMR (150.90 MHz, MeOD, 25 °C): δ = 197.73 (aryl-C=O), 169.46 (benzophenone-C=O), 141.37 (aryl-C),139.24 (aryl-C) 138.42 (aryl-C), 134.15 (aryl-C), 131.06 (2 aryl-C), 130.97 (2 aryl-C), 129.66 (2 aryl-C), 128.49 (2 aryl-C), 101.86 (C-1), 74.87 (C-5), 72.60 (C-3), 72.15 (C-2), 68.64 (C-4), 67.26 (manO*C*H_2CH_2), 62.93 (C-6), 41.07 (manOCH_2*C*H_2) ppm. MALDI-TOF-MS: m/z = 454.3 [M + Na]$^+$. ESI-MS: m/z = 454.12 [M + Na]$^+$. ESI-HRMS: calcd. for $C_{22}H_{25}NNaO_8$ [M + Na]$^+$ 454.1472; found 454.1452.

Lysine Derivative 17: 2′-Aminoethyl α-D-mannoside (**15**) (375 mg, 1.65 mmol), HATU (590 mg, 1.65 mmol), and Fmoc-Lys(Boc)-OH (630 mg, 1.35 mmol) were mixed in a Schlenk flask and dried in vacuo for 15 min. The solid mixture was then dissolved in dry DMF (16 mL) and DIPEA (0.53 mL, 2.70 mmol) was carefully added. This reaction mixture was stirred under an argon atmosphere at room temp. for 18 h, and the solvent was evaporated. Purification of the crude product was accomplished by flash chromatography (ethyl acetate/methanol/water, 6:2:1) to yield the desired product as colorless powder (980 mg, 87%) with R_f = 0.61 (ethyl acetate/methanol/water, 6:2:1), which was directly used in the next step without further characterization.

Biotinylated Lysine Derivative 18: Compound **17** (550 mg, 0.82 mmol) was dissolved in trifluoroacetic acid (80% aq., 20 mL) and stirred at room temp. for 30 min. The solvent was evaporated, and the resulting crude product was then coevaporated with toluene (6 × 10 mL), and neutralized with basic ion exchange resin (Amberlite IRA-402). The desired deprotected product (R_f = 0.19; ethyl acetate/methanol/water, 6:2:1) was obtained as a colorless powder. (+)-Biotin (208 mg, 0.85 mmol) and HATU (316 mg, 0.81 mmol) were added to the crude amine (468 mg, 0.81 mmol), and the solid mixture was dried in vacuo for 30 min, and then dissolved in dry DMF (20 mL). DIPEA (0.45 mL, 2.25 mmol) was carefully added and the resulting reaction mixture was stirred under an argon atmosphere at room temp. for 18 h. The solvent was evaporated, and the crude product was purified by flash chromatography (ethyl acetate/methanol/water, 6:2:1). After lyophilization, compound **18** resulted as a colorless, fluffy powder (496 mg, 69% over two reaction steps). R_f = 0.40 (ethyl acetate/methanol/water, 6:2:1). ^{1}H NMR (500 MHz, MeOD, 25 °C): δ = 7.84 (d, J = 7.5 Hz, 2 H, Fmoc-H), 7.72 (dd, J = 10.4, 7.7 Hz, 2 H, Fmoc-H), 7.43 (t, J = 7.4 Hz, 2 H, Fmoc-H), 7.34 (td, J = 7.5, 1.2 Hz, 2 H, Fmoc-H), 4.81 (d, J = 1.4 Hz, 1 H, 1-H), 4.44 (dd, J = 9.0, 5.7 Hz, 1 H, Fmoc-C*H*CH_2), 4.38 (ddd, J = 7.9, 4.2, 0.8 Hz, 1 H, biotin-NHC*H*CHalkyl), 4.18 (dd, J = 7.9, 4.5 Hz, 1 H, biotin-NHC*H*CH_2S), 3.72 (dd, J = 7.2, 3.9 Hz, 1 H, 6a-H), 3.71 (dd, J = 2.8, 1.4 Hz, 1 H, 2-H), 3.68 (dd, J = 6.8, 4.1 Hz, 1 H, 6b-H), 3.62

(dd, *J* = 8.9, 3.4 Hz, 1 H, 3-H), 3.57 (dd, *J* = 11.7, 5.7 Hz, 1 H, manOC*H*HCH$_2$), 3.50 (t, *J* = 9.2 Hz, 1 H, 4-H), 3.47 (dd, *J* = 11.5, 5.1 Hz, 1 H, manOCH$_2$C*H*H), 3.45 (dd, *J* = 11.6, 4.7 Hz, 1 H, manOCH$_2$CH*H*), 3.40 (dd, *J* = 11.6, 5.2 Hz, 1 H, manOCH*H*CH$_2$), 3.31 (dd, *J* = 6.4, 4.3 Hz, 1 H, 12a-H), 3.30 (dd, *J* = 7.7, 1.1 Hz, 1 H, biotin-CH*H*), 3.29 (ddd, *J* = 10.5, 6.1, 4.0 Hz, 1 H, 5-H), 3.28 (dd, *J* = 6.3, 4.3 Hz, 1 H, 12b-H), 3.26 (dd, *J* = 4.5, 1.0 Hz, 1 H, biotin-C*H*H), 3.17 (ddd, *J* = 10.3, 7.9, 4.5 Hz, 1 H, biotin-NHCHC*H*alkyl), 3.11 (dd, *J* = 9.0, 6.6 Hz, 1 H, 13a-H), 3.09 (ddd, *J* = 9,0, 7.0, 2.1 Hz, 1 H, 10-H), 3.06 (dd, *J* = 9.3, 6.0 Hz, 1 H, 13b-H,), 2.80 (dd, *J* = 12.8, 5.0 Hz, 1 H, Fmoc-CHC*H*H), 2.59 (d, *J* = 12.7 Hz, 1 H, Fmoc-CHCH*H*), 2.08 (t, *J* = 7.4 Hz, 2 H, 16a/b-H), 1.85 (dd, *J* = 10.2, 5.8 Hz, 1 H, 14a-H), 1.82 (dd, *J* = 10.0, 5.7 Hz, 1 H, 14b-H), 1.78 (dd, *J* = 7.9, 3.7 Hz, 1 H, 17a-H), 1.76 (dd, *J* = 7.4, 3.9 Hz, 1 H, 17b-H), 1.61 (dd, *J* = 7.7, 6.1 Hz, 1 H, 19a-H), 1.59 (dd, *J* = 7.5, 3.2 Hz, 1 H, 19b-H), 1.53 (dd, *J* = 15.5, 7.9 Hz, 1 H, 18a-H), 1.46 (dd, *J* = 9.0, 7.2 Hz, 1 H, 11a-H), 1.43 (dd, *J* = 7.1, 2.5 Hz, 1 H, 11b-H), 1.39 (dd, *J* = 15.7, 7.5 Hz, 1 H, 18b-H) ppm. ^{13}C NMR (125.75 MHz, MeOD, 25 °C): *δ* = 176.03 (C-9), 175.09 (C-15), 166.10 [(NH)$_2$-C=O], 158.47 (Fmoc-C=O), 151.37 (2 Fmoc-C), 148.57 (2 Fmoc-C), 128.84 (2 Fmoc-C), 128.21 (2 Fmoc-C), 126.26 (2 Fmoc-C), 120.96 (2 Fmoc-C), 101.70 (C-1), 74.77 (C-5), 72.60 (C-3), 72.11 (C-2), 68.74 (C-4), 67.94 (C-6), 67.11 (manO*C*H$_2$CH$_2$), 63.38 (biotin-NH*C*HCH$_2$), 62.98 (manOCH$_2$*C*H$_2$), 61.64 (biotin-NH*C*HCHalkyl), 57.01 (C-10), 56.76 (biotin-NHCH*C*Halkyl), 55.57 (Fmoc-CH), 41.04 (biotin-CH$_2$), 40.32 (C-14), 36.79 (C-12), 33.00 (C-13), 30.02 (C-16), 29.75 (C-17), 29.47 (C-18), 26.86 (C-19), 24.35 (C-11) ppm. MALDI-TOF-MS: *m*/*z* = 822.9 [M + Na]$^+$, 838.9 [M + K]$^+$. ESI-HRMS: calcd. for $C_{39}H_{53}N_5NaO_{11}S$ [M + Na]$^+$ 822.92; found 822.34.

Benzophenone-Substituted Biotinylated Lysine Derivative 19: Compound **18** (279 mg, 0.35 mmol) was dissolved in dry DMF (8 mL) and piperidine (2 mL), and the resulting solution was stirred under an argon atmosphere at room temp. for 30 min. The mixture was then concentrated, and the crude product (280 mg, 0.35 mmol) was dissolved in DMF (12 mL) and added to a mixture of 4-benzoylbenzoic acid (68.0 mg, 0.30 mmol) and HATU (130 mg, 0.35 mmol), which had been dried in vacuo for 30 min. To the resulting solution, DIPEA (0.7 mL, 0.70 mmol) was carefully added and the mixture was stirred at room temp. under an argon atmosphere for 20 h. The solvent was evaporated and the crude product was purified by two subsequent flash chromatographic separations on RP-silica gel (ethyl acetate/methanol, 1:1; ethyl acetate/methanol/water, 6:2:1) to obtain photolabile derivative **19** (110 mg, 40% from **15**) as light yellow crystals. R_f = 0.33 (ethyl acetate/methanol/water, 6:2:1). M.p. 134 °C. $[\alpha]_D$ = + 17.8 (*c* = 2.29, H$_2$O). ^{1}H NMR (500 MHz, MeOD, 25 °C): *δ* = 7.92 (ddd, *J* = 8.6, 1.9, 1.0 Hz, 2 H, benzophenone-*m*-H), 7.74 (ddd, *J* = 8.6, 1.9, 1.0 Hz, 2 H, benzophenone-*o*-H), 7.70 (dd, *J* = 8.4, 1.3 Hz, 2 H, benzophenone-*o*-H), 7.57 (m$_c$, *J* = 7.5, 1.5 Hz, 1 H, benzophenone-*p*-H), 7.45 (m$_c$, *J* = 7.6, 1.6, 0.4 Hz, 2 H, benzophenone-*m*-H), 4.68 (d, *J* = 1.6 Hz, 1 H, 1-H), 4.44 (dd, *J* = 9.0, 5.7 Hz, 1 H, 10-H), 4.38 (ddd, *J* = 7.9, 5.0, 0.8 Hz, 1 H, biotin-NHC*H*CH$_2$S), 4.18 (dd, *J* = 7.9, 4.5 Hz, 1 H, biotin-NHC*H*CHalkyl), 3.72 (dd, *J* = 7.2, 6.9 Hz, 1 H, 14a-H), 3.71(dd, *J* = 3.4, 1.6 Hz, 1 H, 2-H), 3.68 (dd, *J* = 6.4, 4.1 Hz, 1 H, 6a-H), 3.62 (dd, *J* = 8.9, 3.4 Hz, 1 H, 3-H), 3.57 (dd, *J* = 13.7, 5.7 Hz, 1 H, manOCH*H*CH$_2$), 3.50 (t, *J* = 9.2 Hz, 1 H, 4-H), 3.41 (dd, *J* = 7.0, 6.7 Hz, 1 H, 14b-H), 3.40 (dd, *J* = 6.6, 4.2 Hz, 1 H, 6b-H), 3.29 (ddd, *J* = 9.2, 6.4, 4.3 Hz, 1 H, 5-H), 3.11 (dd, *J* = 13.8, 7.9 Hz, 1 H, manOC*H*HCH$_2$), 3.07 (ddd, *J* = 10.3, 5.9, 4.5 Hz, 1 H, biotin-NHCHC*H*alkyl), 2.80 (dd, *J* = 12.8, 5.0 Hz, 1 H, manOCH$_2$C*H*H), 2.58 (d, *J* = 12.7 Hz, 1 H, manOCH$_2$CH*H*), 1.83 (dd, *J* = 13.8, 6.2 Hz, 1 H, biotin-NHCHC*H*HS), 1.82 (dd, *J* = 6.1, 3.5 Hz, 1 H, 16a-H), 1.81 (dd, *J* = 9.8, 5.1 Hz, 1 H, 19b-H), 1.78 (dd, *J* = 6.2, 3.3 Hz, 1 H, 16b-H), 1.76 (dd, *J* = 9.3, 4.5 Hz, 1 H, 18a-H), 1.74 (dd, *J* = 9.2, 4.5 Hz, 1 H, 13a-H), 1.73 (dd, *J* = 10.1, 4.6 Hz, 1 H, 19a-H), 1.61 (dd, *J* = 14.1, 7.6 Hz, 1 H, 17a-H), 1.59 (dd, *J* = 9.8, 8.0 Hz, 1 H, 11a-H), 1.57 (dd, *J* = 13.0, 5.3 Hz, 1 H, biotin-NHCHCH*H*S), 1.55 (dd, *J* = 10.0, 7.7 Hz, 1 H, 11b-H), 1.53 (dd, *J* = 14.9, 7.4 Hz, 1 H, 12a-H), 1.51 (dd, *J* = 9.3, 6.9 Hz, 1 H, 13b-H), 1.47 (dd, *J* = 13.8, 7.8 Hz, 1 H, 17b-H), 1.36 (dd, *J* = 9.7, 6.5 Hz, 1 H, 18b-H), 1.31 (dd, *J* = 15.2, 7.6 Hz, 1 H, 12b-H) ppm. ^{13}C NMR (125.75 MHz, MeOD, 25 °C): *δ* = 197.70 (aryl-C=O), 176.07 (C-15), 174.59 (C-9), 169.37 (benzophenone-C=O) 166.09 [(NH)$_2$-C=O], 141.51 (2 aryl-C), 138.73 (2 aryl-C), 138.37 (aryl-C), 134.19 (aryl-C), 131.09 (2 aryl-C), 130.95 (2 aryl-C), 129.67 (2 aryl-C), 128.78 (2 aryl-C), 101.69 (C-1), 74.75 (C-5), 72.60 (C-2), 72.11 (C-3), 68.78 (C-4), 67.08 (C-6), 63.38 (biotin-NH*C*HCHalkyl), 62.98 (C-14), 61.63 (biotin-NH*C*HCH$_2$), 57.02 (biotin-NHCH*C*Halkyl), 55.72 (C-10), 41.05 (manO*C*H$_2$CH$_2$), 40.42 (manOCH$_2$*C*H$_2$), 39.99 (C-16), 35.79 (C-19), 32.74 (C-11), 30.12 (C-13), 29.76 (biotin-CH$_2$), 29.45 (C-17), 26.86 (C-18), 24.52 (C-12) ppm. UV: λ_{max}(1) = 327.8 nm (*c* = 0.75 mM, doubly distilled H$_2$O), *ε*(1) = 1013.3 L mol^{-1} cm^{-1}, λ_{max}(2) = 290.0 nm (*c* = 0.75 mM, doubly distilled H$_2$O), *ε*(2) = 3613.3 L mol^{-1} cm^{-1}, λ_{max}(3) = 242.8 nm (*c* = 0.75 mM, doubly distilled H$_2$O), *ε*(2) = 4213.3 L mol^{-1} cm^{-1}. MALDI-TOF-MS: *m*/*z* = 808.7 [M + Na]$^+$, 824.7 [M +K]$^+$. ESI-MS: *m*/*z* = 808.32 [M + Na]$^+$. ESI-HRMS: 808.3184 [M + Na]$^+$.

Irradiation Experiments

Evaluation of the Decomposition Time of the Photolabile Residues and Detection of Insertion Products: The experimental setup for the irradiation tests consisted of a UV lamp (Peschl-Consulting Mainz, 150 Watt) and a metal box to exclude external light. A conical glass vial equipped with a septum to assure an inert gas atmosphere was used as the reactor. The distance between the light source and the sample was kept constant at 12 cm. To obtain the optimal wavelength range appropriate glass filters (Schott AG) were attached between the light source and the sample. Sample volumes for photolysis were 5.0 mL and 0.5 mL (Table 1).

To determine the absorption maxima and the decomposition time, sample solutions were prepared by the dissolution of the photolabile compounds in doubly distilled H$_2$O or MeOH at concentrations of 0.1 mM (**12** and **14**), 0.25 mM (**16**) and 0.75 mM (**19**). Photolysis experiments were carried out with UV light at wavelengths ≥320 nm (total sample volume 5.0 mL). UV spectra were recorded prior to irradiation and to monitor the decrease in the absorption maximum upon photolysis; UV spectra were recorded every 5 min during irradiation. The covalent insertion products formed with the respective solvents were detected by MALDI-TOF-MS or ESI-MS:

*Photolysis of Diazirine **14** in MeOH:* The insertion product of the generated carbene and MeOH was formed. MALDI-TOF-MS: *m*/*z* = 381.4 [M – N$_2$ + MeOH]$^+$. ESI-MS: *m*/*z* = 405.11 [M – N$_2$ + MeOH + Na]$^+$.

*Photolysis of Diazirine **14** in Doubly Distilled H$_2$O:* The insertion product of the generated carbene and water was formed. MALDI-TOF-MS: *m*/*z* = 367.4 [M – N$_2$ + H$_2$O]$^+$. ESI-MS: *m*/*z* = 391.10 [M – N$_2$ + H$_2$O + Na]$^+$.

*Photolysis of Azide **12** in MeOH:* The insertion product of the generated nitrene and MeOH was formed. ESI-MS: *m*/*z* = 410.17 [M – N$_2$ + MeOH + Na]$^+$.

*Photolysis of Azide **12** in Doubly Distilled H$_2$O:* No insertion products were discernable.

Irradiation Experiments with Various Amino Acids: Irradiation experiments were carried out in doubly distilled H_2O in the case of PALs **14**, **16**, and **19** and in MeOH for**12**. The total sample volume was 0.5 mL with PAL/amino acid ratios of 1:2, 1:5, 1:10, and 2:1. The concentration of each PAL was chosen as 0.1 mM; the concentrations of the amino acids were 0.2 mM, 0.5 mM, 1.0 mM, and 0.05 mM. The samples were irradiated for 30 min (**12**), 10 min (**14**), or 60 min (**16** and **19**). After photolysis, the samples were concentrated by ZipTipping® to allow for MS analysis (Table 2).

*Insertion Products Found with Azide **12**:* MALDI-TOF-MS: *m/z* = 707.3 $[C_{34}H_{36}F_4N_2O_{10} - H]^+$, covalent adduct with Fmoc-Ile-OH.

*Insertion Products Found with Diazirine **14**:* MALDI-TOF-MS: *m/z* = 568.5 $[C_{24}H_{34}F_3NO_{11} - H]^+$, covalent adduct with Boc-Thr-OH; 523.3 $[C_{21}H_{31}F_3N_4O_8 - H]^+$, covalent adduct with H-Arg-OH; 631.4 $[C_{29}H_{37}F_3N_2O_{10} + H]^+$, covalent adduct with Z-Lsy-OH.

*Insertion Products Found with Benzophenone **16**:* MALDI-TOF-MS: *m/z* = 710.8 $[C_{36}H_{45}N_3O_{12} - H]^+$, covalent adduct with Z-Lys-OH.

*Insertion Products Found with Benzophenone **19**:* MALDI-TOF-MS: *m/z* = 1066.3 $[C_{52}H_{71}N_7O_{15}S]$, covalent adduct with Z-Lys-OH.

Irradiation Experiments in the Presence of Model Peptides Angiotensin II and PTHIKWGD and Pentaglycine: To investigate the potential of PALs **12**, **14**, **16** and **19** in the presence of peptides, sample mixtures of the various PALs with a 10-fold excess of investigated peptide in doubly distilled H_2O (total sample volume of 0.5 mL) were irradiated with UV light ≥320 nm for 30 min (**12**), 10 min (**14**), or 60 min (**16** and **19**). The concentrations were chosen as 0.1 mM for each PAL and 1.0 mM for each peptide. For work-up, the ZipTip® technique[33] was used prior to MALDI-TOF-MS analysis to reduce the content of cations such as Na^+ and K^+ and to increase the concentration of the insertion products in the respective sample (Table 3).

*Irradiation of Azide **12** with Angiotensin II:* MALDI-TOF-MS: *m/z* = 1415.5 $[C_{63}H_{83}F_4N_{15}O_{18} + H]^+$. ESI-MS: *m/z* = 707.29.

*Irradiation of Azide **12** with PTHIKWGD:* MALDI-TOF-MS: *m/z* = 1308.7 $[C_{57}H_{77}F_4N_{13}O_{18}]^+$.

*Irradiation of Diazirine **14** with Angiotensin II:* MALDI-TOF-MS: *m/z* = 1396.6 $[C_{65}H_{88}F_3N_{13}O_{18} + H]^+$. ESI-MS: *m/z* = 698.82 $[C_{65}H_{88}F_3N_{13}O_{18} + H]^+$.

*Irradiation of Diazirine **14** with PTHIKWGD:* MALDI-TOF-MS: *m/z* = 1326.6 $[C_{59}H_{81}F_3N_{12}O_{18} + Na]^+$. ESI-MS: *m/z* = 671.29 $[C_{59}H_{81}F_3N_{12}O_{18} + K]^+$.

*Irradiation of Benzophenone **16** with Pentaglycine:* MALDI-TOF-MS: *m/z* = 733.8 $[C_{32}H_{42}N_6O_{14} - H]^+$.

*Irradiation of Biotinylated PAL **19** with Angiotensin II:* MALDI-TOF-MS: *m/z* = 1848.2 $[C_{88}H_{122}N_{18}O_{24}S]^+$.

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