The Staudinger Ligation

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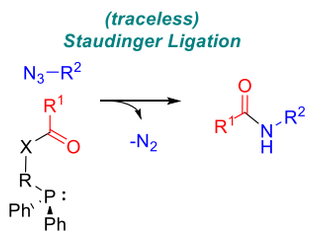
ABSTRACT: While the Staudinger reaction has first been described a hundred years ago in 1919, the ligation reaction became one of the most important and efficient bioconjugation techniques in the 1990s and this century. It holds the crucial characteristics for bioorthogonal chemistry: biocompatibility, selectivity, and a rapid and high yielding turnover for a wide variety of applications. In the past years, it has been used especially in chemical biology for peptide/protein synthesis, posttranslational modifications, and DNA labeling. Furthermore, it can be used for cell surface engineering, development of microarrays, and drug delivery systems. However, it is also possible to use the reaction in synthetic chemistry for general formation of amide bonds. In this review, the three major types, traceless and nontraceless Staudinger Ligation as well as the Staudinger phosphite reaction, are described in detail. We will further illustrate each reaction mechanism and describe characteristic substrates, intermediates, and products. In addition, not only its advantages but also stereochemical aspects, scope, and limitations, in particular side reactions, are discussed. Finally, the method is compared to other bioorthogonal labeling methods.



R^1 = e.g. peptides, proteins, fatty acids, fluorophores,biotin, FLAG, radionuclides etc.
R^2 = e.g. proteins, peptides, lipids

materials and biology

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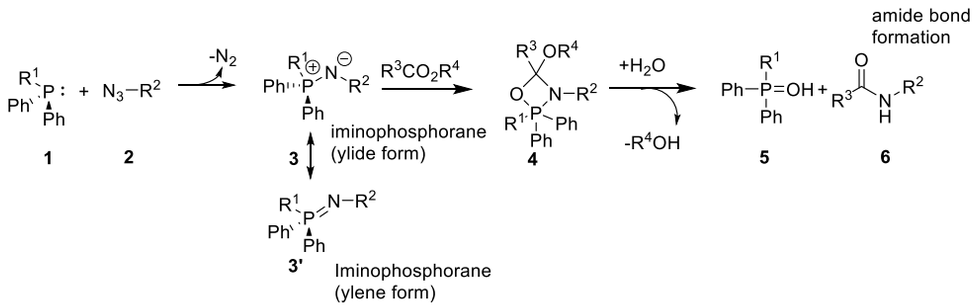
1. INTRODUCTION

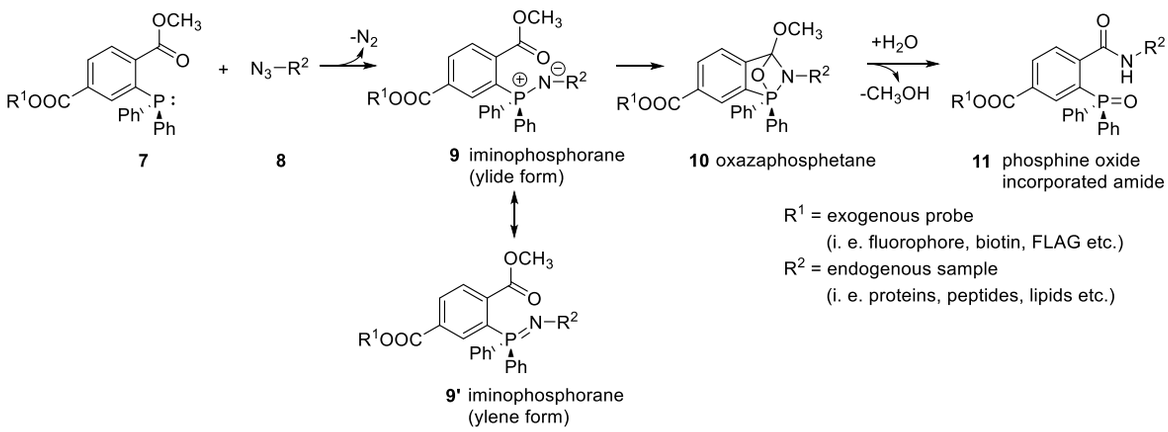
This review focuses on the Staudinger reaction for selective and mild bioorthogonal conjugation reactions, also referred as ligations reactions. The Staudinger Ligation is an important method for bioconjugation as amides are inherent in biological systems and the ligation has been shown to connect biologically relevant components very efficiently. It is used for a wide variety of applications, e.g., peptide or protein synthesis, post translational modification, cell surface engineering, labeling with dyes, labeling of glass surfaces, radiochemical/radio pharmaceutical labeling, and coating of microarrays.[1−3]

The Staudinger Ligation is a bioconjugation method using the Staudinger reaction,[4] which goes back to investigations of Staudinger in 1919 as the key step. Of particular interest is the application of the Staudinger Ligation, an intramolecular click

Scheme 1. Principle of the Staudinger Ligation



Scheme 2. Nontraceless Staudinger Ligation



type[5,6] reaction, in labeling molecules or biological entities in vitro. Thus, this reaction is nowadays regarded as one of the most important bioconjugation techniques, as it combines the advantages of bioorthogonality and selectivity, simultaneously being rapid and high yielding. Many other known reactions of this type as, e.g., Diels–Alder reactions are limited in terms of the key chemoselective ligation reactions and their application to chemical biology.[2,7,8]

The principle of this ligation is shown in Scheme 1:[9,10] In the first step, which is the so called Staudinger reaction, a phosphane **1** is reacted with an azide **2**, yielding an iminophosphorane **3** (in the ylide form **3** or in the ylene form **3′**). These intermediately formed iminophosphoranes, which react with electrophiles, are widely used in different research areas since the late 1990s.[11−17] The following conversion of the iminophosphorane **3′** with carboxylic acid derivatives yields amides **6** via the formation of a carbon–nitrogen bond and is then known as Staudinger Ligation. There are three major conjugation variants regarding the Staudinger Ligation,[1,3,5,18−75] which are discussed in detail in the following: (1) the nontraceless Staudinger Ligation, (2) the traceless Staudinger Ligation, and (3) the Staudinger phosphite reaction, which gives chemoselective access to phosphoramidates.

Solely intermolecular reactions are not considered as ligation methods, however, in Table 6, we list some examples being named "Staudinger Ligation" (which was in fact discovered by the late Leopold Horner).[76] In these cases, the carboxylic acid has to be preactivated, e.g., as benzotriazoyl esters or with activation reagents like DCC or EDS to ensure high reactivity. A catalytic variant using silanes as reducing agent is known.[77] The reaction of iminophosphoranes with acid chlorides is in fact much older.[78] Alternatively, disulfides or diselenides can be used.[79] In Table 6, we list a related reaction involving phosphites.

2. MECHANISM AND STEREOCHEMISTRY

2.1. Nontraceless Staudinger Ligation

The first variant of Staudinger Ligation is the nontraceless Staudinger Ligation, also known as the nontraceless azide–phosphane ligation, where a carboxamide is formed while incorporating the phosphane reagent. This bioorthogonal reaction was introduced in 2000 by Bertozzi and co workers.[9] In Scheme 2, an exemplary reaction is shown, using an *ortho* phosphane terephthalic acid derivative **7** and an azide as starting materials.[9,10] As stated previously, after formation of an iminophosphorane **9** a subsequent cyclization yields oxazaphos phetane **10** as an initial intermediate. Eventually, the desired amide **11** arises through spontaneous hydrolysis in aqueous media, into which the phosphane oxide byproduct is incorporated.[9,80]

In the following tables, two different ligation types along with the Staudinger Ligation yielding cyclic structures and other related azide–phosphane ligations are covered. We will provide as much information as possible because in biological systems, exact chemical structures and/or conditions are not always rigorously described.

The entries are organized by number of carbon atoms of the phosphane (and then grouped by publication), and it will cover examples up to beginning of 2020 (for not or only partly covered examples, see refs 81–149).

For natural amino acids, the three letter code is used (unless the starting materials are explicitly drawn) and the nonracemic ones are in general L. Sugars are represented as molecular formulas (with the exception of glycopeptides) (Table 1).

Table 1. Classical (Nontraceless) Staudinger Ligation[5,8−10,80,117,120,132,138,144,150−250]

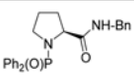
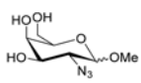
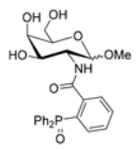
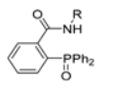
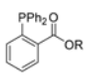
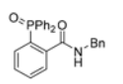
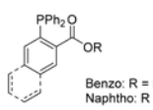
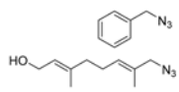
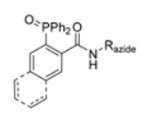
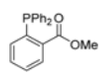
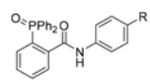
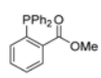
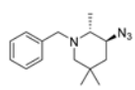
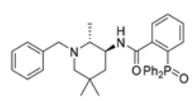
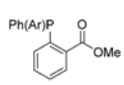
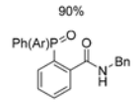
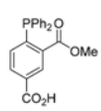
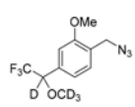
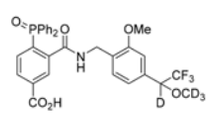
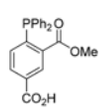
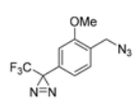
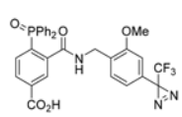
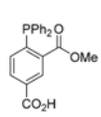
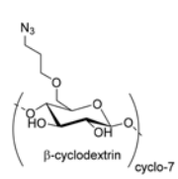
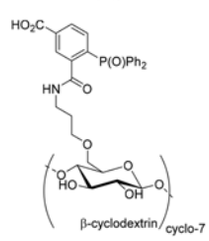
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{19}			various conditions, 30 min	 up to 95%	150
C_{20}			3:1 THF/H_2O, 2 h, rt		9
C_{20}		$Ph(CH_2)_4N_3$ $PhCH_2OCOCH_2N_3$ $PhCH_2NHCOCH_2N_3$ $Ph(CH_2)_4NHCOCH_2N_3$ $PhCH_2NHCO(CH_2)_2N_3$ $PhCH_2NMeCOCH_2N_3$	aq. MeCN, rt	 n/a (kinetic study)	151
C_{20} C_{21} C_{22} C_{23}	 R = Me, Et, iPr, tBu		CD_3CN, rt	 n/a (kinetic investigations)	152
C_{20} C_{21} C_{24}	 Benzo: R = Me, Et Naphtho: R = Et		CD_3CN, rt	 n/a (mass spectroscopical investigations)	153
C_{20}		 R = H, OMe, NO_2	CD_3CN, rt	 R = H, OMe, NO_2 n/a (kinetic investigations)	154
C_{20}			MeCN/H_2O, 37 °C, 48 h	 90%	155
C_{20} C_{21} C_{22} C_{23}	 Ar = Ph, p-$Me_2NC_6H_4$, p-$MeOC_6H_4$, p-HOC_6H_4, p-MeC_6H_4, p-BrC_6H_4, p-$O_2NC_6H_4$		CD_3CN, rt	 n/a (kinetic investigations)	154
C_{21}			4:1 THF/H_2O, 24 h	 39% (2 steps)	156
C_{21}			4:1 THF/H_2O, 24 h	 54%	156
C_{21}		 β-cyclodextrin cyclo-7	15:1 DMF/H_2O, 40 °C	 β-cyclodextrin cyclo-7 95%	157

Table 1. continued

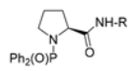
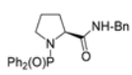
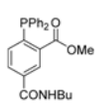
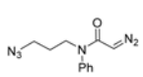
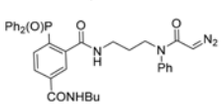
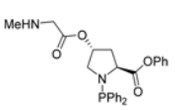
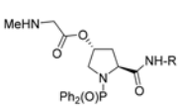
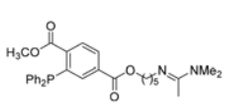
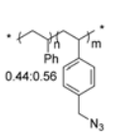
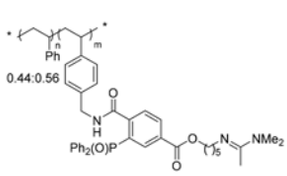
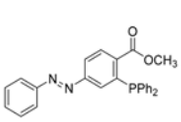
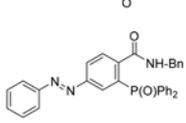
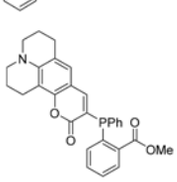
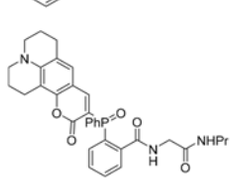
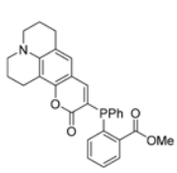
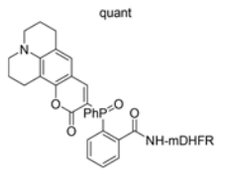
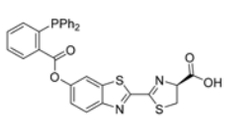
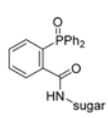
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{23}		R-N_3 R = Bn Phe-NHBn Leu-NHBn Lys(Cbz)-NHBn PheGly-NH_2 Lys(Cbz)Phe-OMe LeuPhe-OMe LeuLysPhe-OMe LeuLys(Boc)Phe-OH	DMF, buffer (pH 7.4)	 R = Phe-NHBn 91% Leu-NHBn 92% Lys(Cbz)-NHBn 91% PheGly-NH_2 94% Lys(Cbz)Phe-OMe 90% LeuPhe-OMe 89% LeuLysPhe-OMe 78% LeuLys(Boc)Phe-OH 90%	150
C_{23}			various conditions, 30 min	 up to 95%	150
C_{25}			THF/H_2O	 70%	138
C_{26}		R-N_3 R = Bn Leu-NHBn LeuPhe-OMe	3:1 DMF/buffer (pH 7.4), 30 min	 R = Bn 91% Leu-NHBn 86% LeuPhe-NHBn 83%	150
C_{25} C_{26}	 R = Ph, p-MeC_6H_4, p-$MeOC_6H_4$, p-ClC_6H_4, p-FC_6H_4, p-$O_2NC_6H_4$		CD_3CN, rt	 n/a (kinetic investigations)	154
C_{26}			—		158
C_{26}			—		159
C_{29}			3:1 MeCN/H_2O	 quant	160
C_{29}		murine dihydrofolate reductase, mDHFR-N_3	3:1 MeCN/H_2O	 quant	160
C_{30}		Sugar-N_3	n/a	 -	161

Table 1. continued

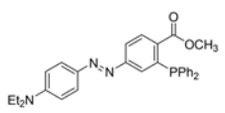
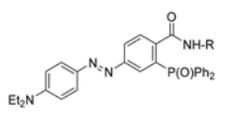
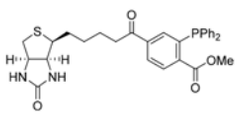
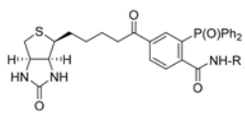
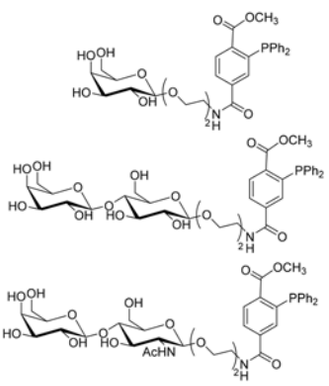
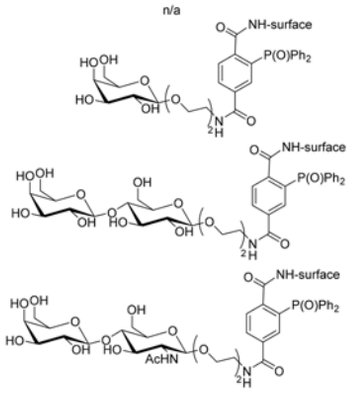
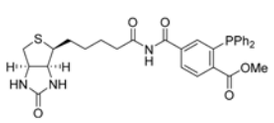
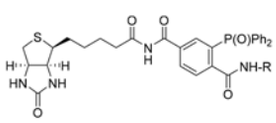
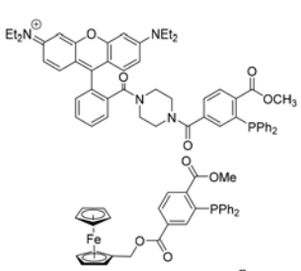
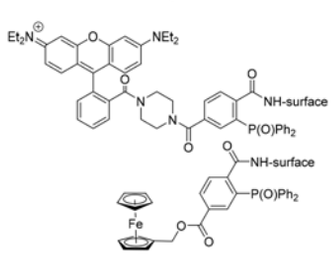
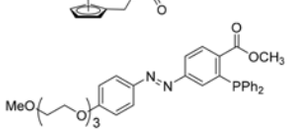
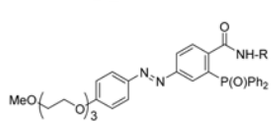
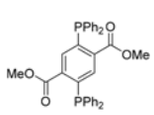
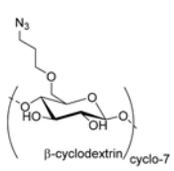
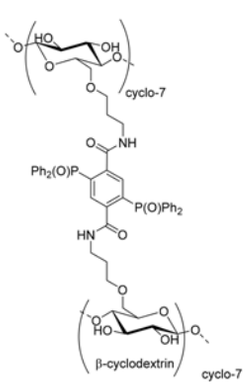
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{30}		Bn-N_3 surface-N_3 peptide-N_3	e.g. H_2O, MeCN, $CHCl_3$		159
C_{30}		azido 4-hydroxynonenal azido-palmitate analogue	n/a		162,163
				n/a	
C_{31} C_{37} C_{39}		surface-N_3	n/a		164
				n/a	
C_{31}		azido diketone	n/a		165
				n/a	
C_{32} C_{53}		surface-N_3			166
C_{33}		R-N_3 R = Bn, surface, protein			159
C_{34}			DMF/$CHCl_3$/ H_2O, 48 h		157
				62%	

Table 1. continued

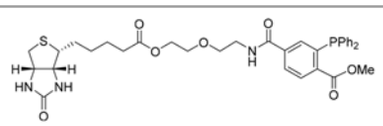
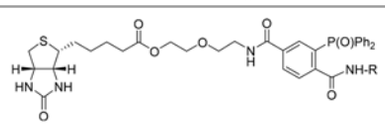
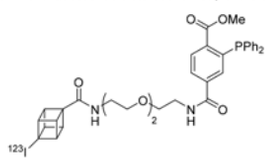
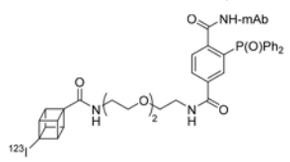
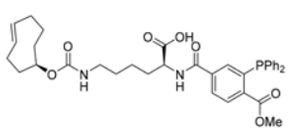
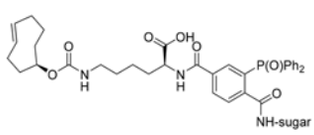
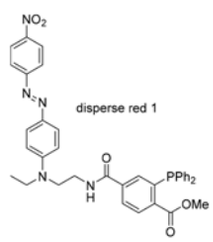
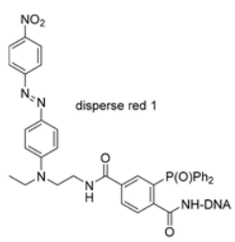
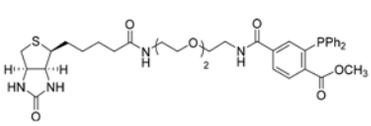
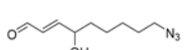
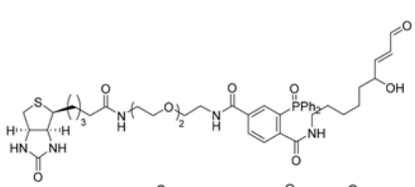
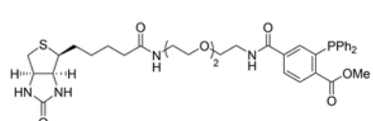
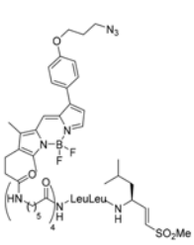
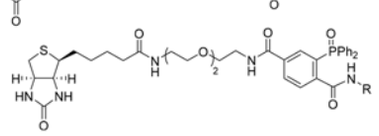
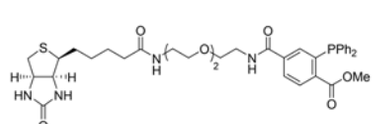
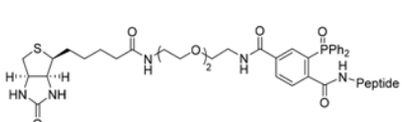
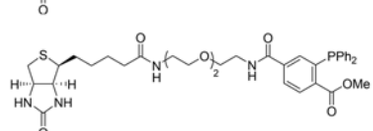
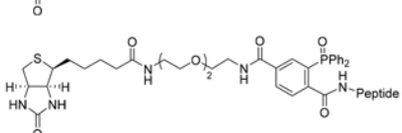
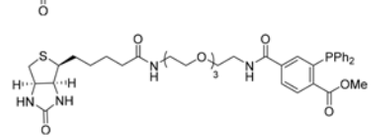
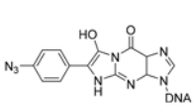
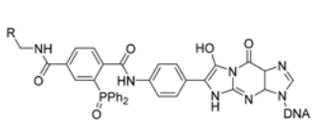
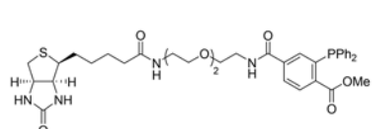
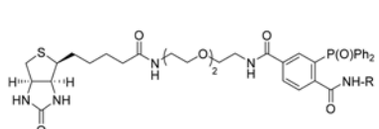
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{35}		carboxymethyl-dextran surface			164
	Comment: Questionable stereochemistry of the biotin			Comment: Questionable stereochemistry of the biotin	
C_{36}		mAb-N_3			167
C_{36}		sugar-N_3	*in vivo*		168
C_{37}		DNA-N_3			169
C_{37}			37 °C, cells, 2 x 1 h		162
C_{37}					170,
C_{37}		Peptide-N_3			171
C_{37}		Peptide-N_3			172
C_{39}	 Comment: There might be an error in the publication for the structure. PEG-3Biotin is drawn	 (in situ formed)			173
C_{37}		Various probes, Sugar-N_3			174,175,176 177,178

Table 1. continued

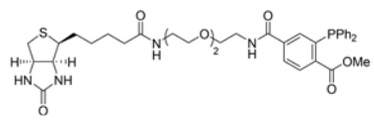
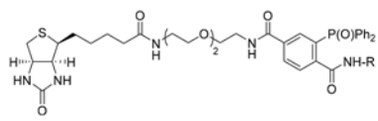
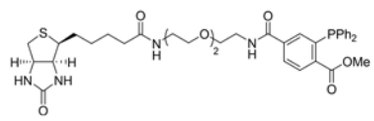
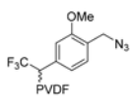
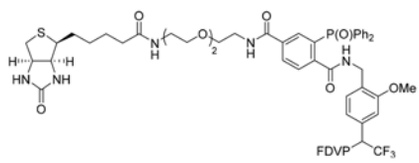
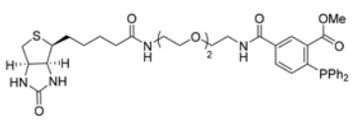
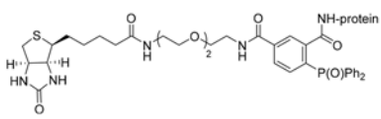
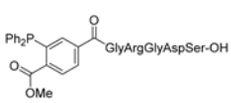
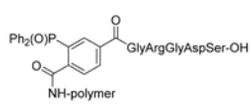
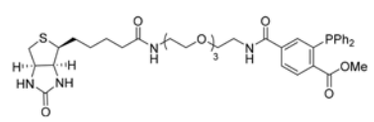
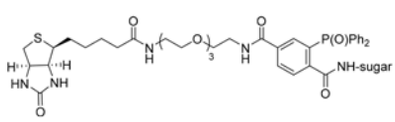
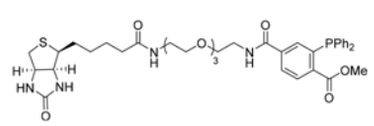
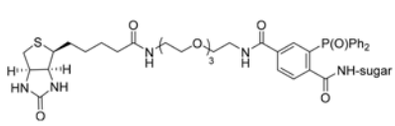
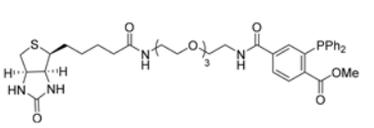
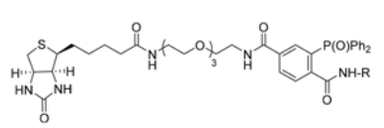
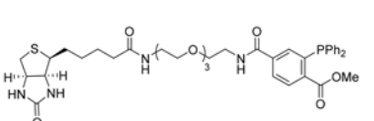
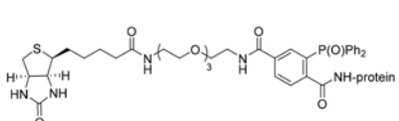
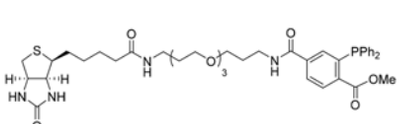
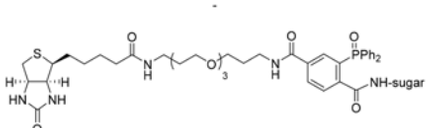
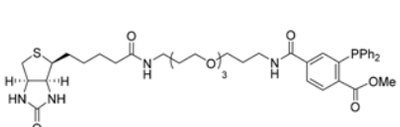
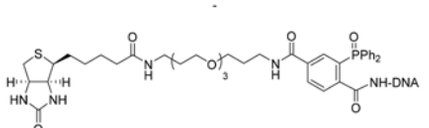
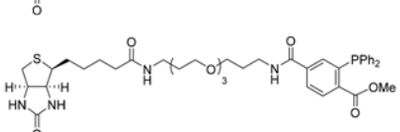
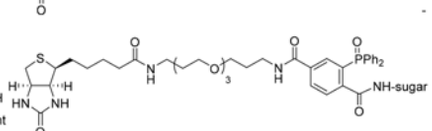
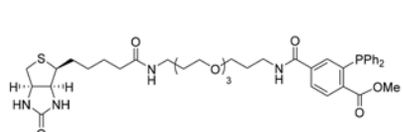
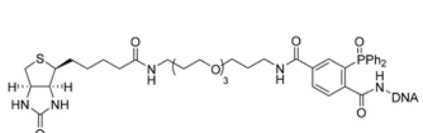
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{37}		Various probes			179
C_{37}					156
C_{37}		Modified protein	n/a		180
C_{38}		polymer-N_3			181
C_{39}		Sugar-N_3	75 μM, in 0.2:1.7:98 DMSO:EtOH:HBSF), or solvent vehicle, for 20 min at 37 °C		182
C_{39}		Sugar-N_3	rt, overnight		183
C_{39}		Various probes	n/a		117,120,184, 185,186-188
C_{39}		Modified protein	n/a		189
C_{41}		Sugar-N_3	37 °C		9
C_{41}		DNA-N_3	3:1 aq. Buffer, DMF or EtOH 60 °C, 12 h		190,191
C_{41}		Sugar-N_3	75 μM, in 0.2:1.7:98 DMSO:EtOH:HBSF), or solvent vehicle, for 20 min at 37 °C		192
C_{41}		DNA-N_3	60 °C, 12 h		193

Table 1. continued

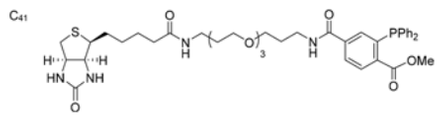
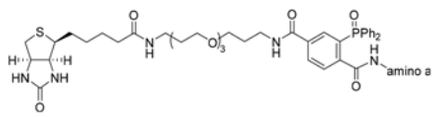
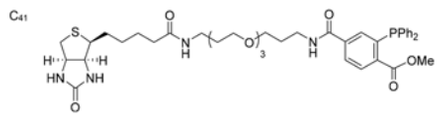
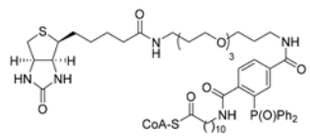
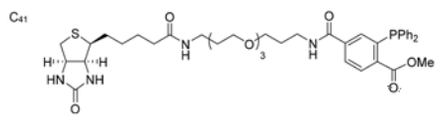
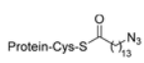
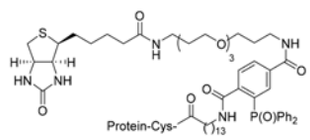
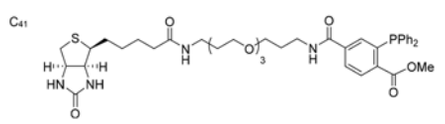
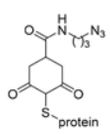
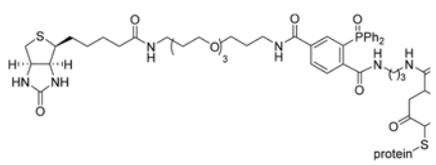
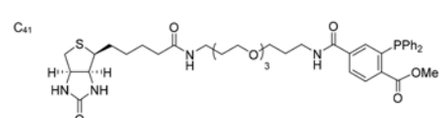
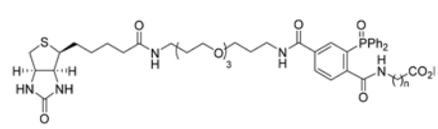
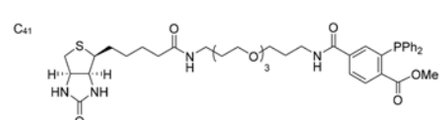
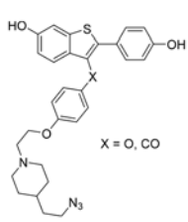
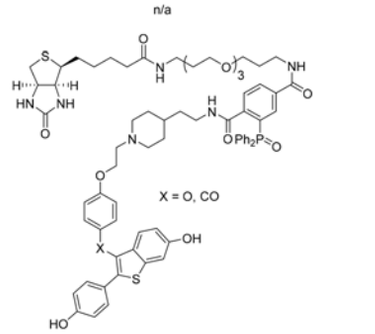
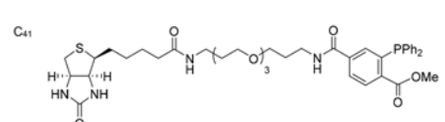
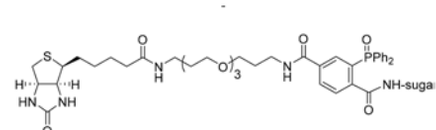
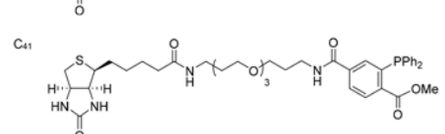
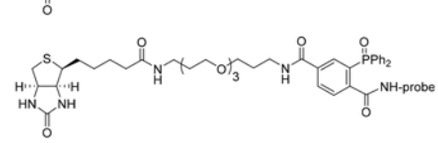
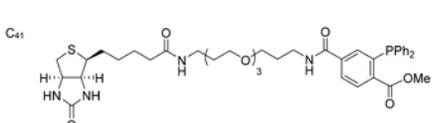
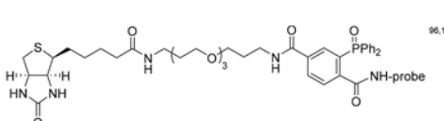
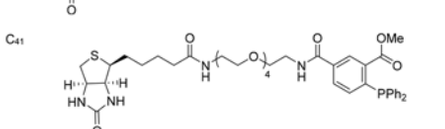
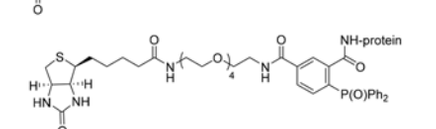
Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
	Cell lysates	37 °C, 2 h		194,195
		n/a	 n/a	196
		n/a	 n/a	163,197
		buffer, 37 °C, 2 h	 n/a	198
	 *n* = 11, 14	n/a	 n/a	199
		n/a	 -	200
	azidoglycosylated nup62	37 °C		201
	azido-dodecanoyl-CoA	37 °C		202
	Various probes			96,190,203-210
	Modified protein	n/a	 -	211

Table 1. continued

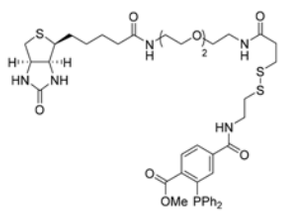
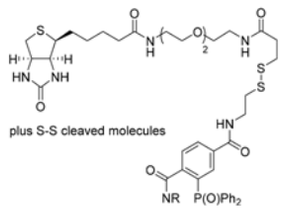
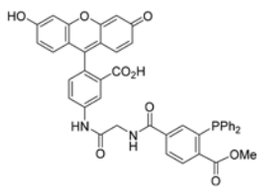
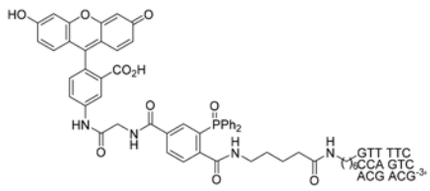
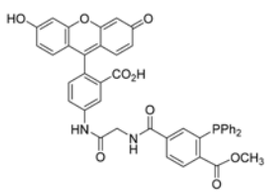
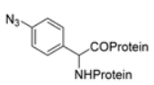
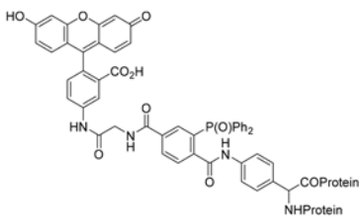
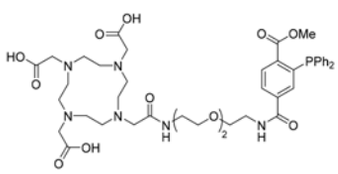
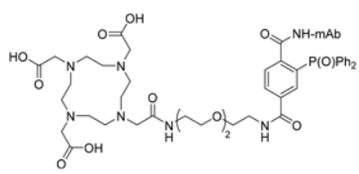
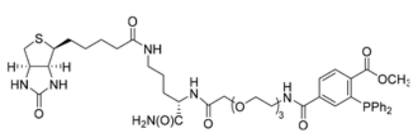
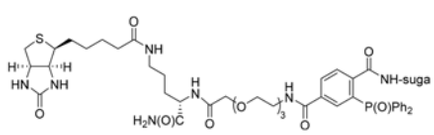
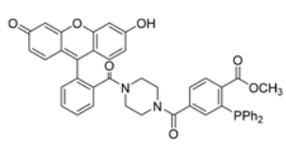
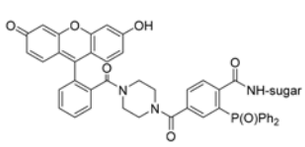
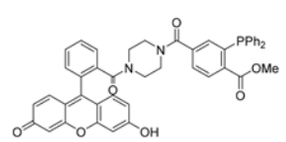
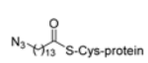
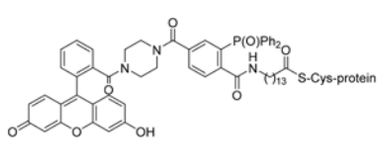
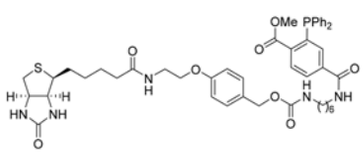
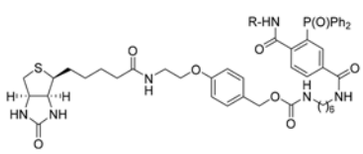
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{42}		Azides		 plus S-S cleaved molecules	212
C_{43}			DMF, buffer, 12 h, rt		213
C_{43}			PBS, pH 7.4, rt, 16 h		214
C_{43}		mAb-N_3			167
C_{44}		Sugar-N_3	37 °C, 2 h		215, 216
C_{45}		Sugar-N_3			217, 218
C_{45}					163,197
C_{47}		Different probes		 n/a	219

Table 1. continued

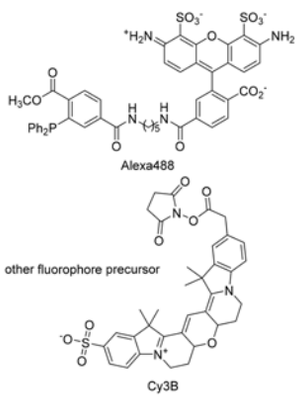
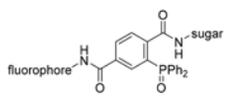
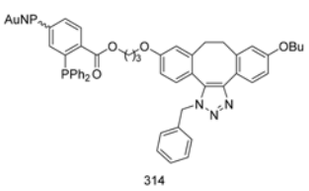
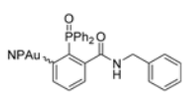
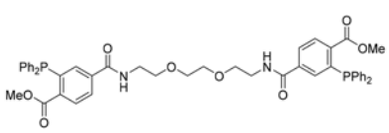
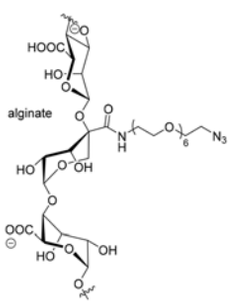
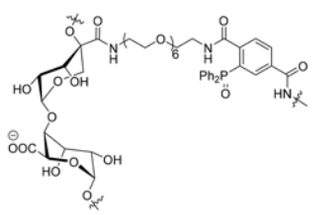
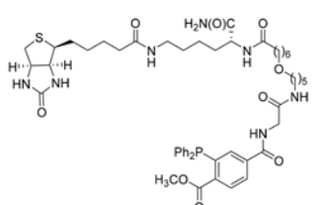
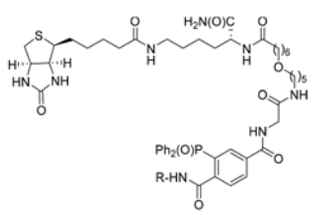
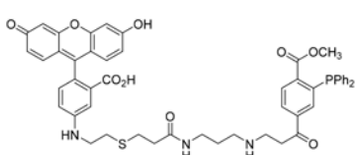
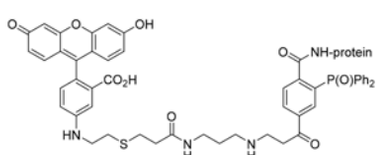
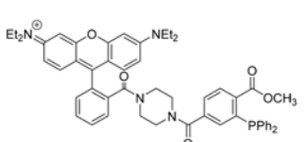
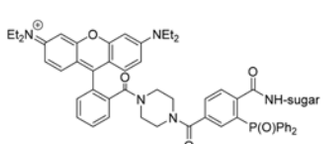
Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{47} C_n C_n 	Sugar-N_3			132
C_{47} 	BnN_3	H_2O CH_2Cl_2/CH_3OH		144
C_{48} 				220
C_{51} 	Sugar-N_3	37 °C, 2 h		215, 216
C_{51} 	protein-N_3			221,222
C_{53} 	Sugar-N_3			217, 218

Table 1. continued

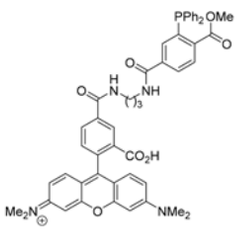
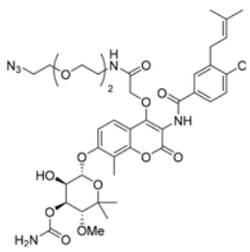
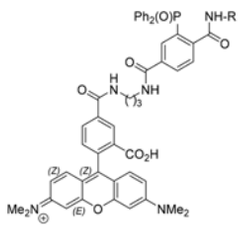
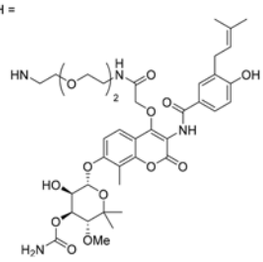
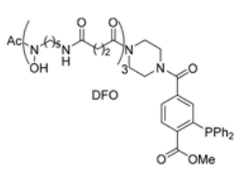
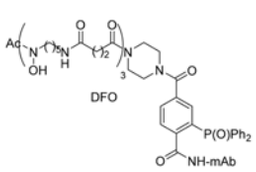
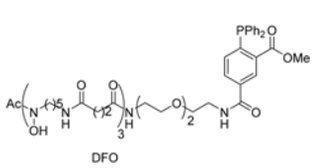
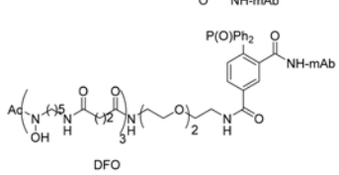
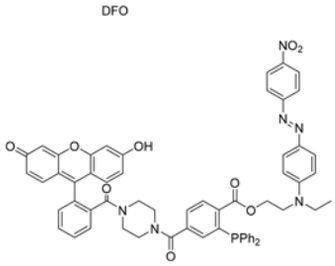
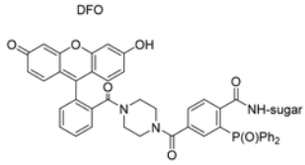
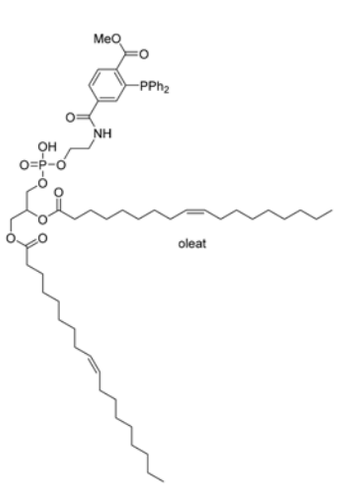
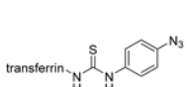
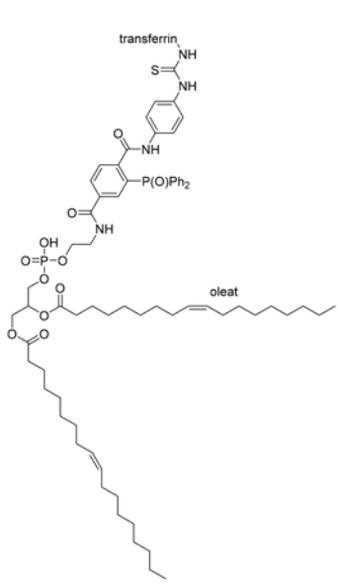
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{53}				 RNH = 	223
C_{54}		mAb-N_3			167
C_{56}		mAb-N_3			167,224
C_{60}		Sugar-N_3			217
C_{62}					225

Table 1. continued

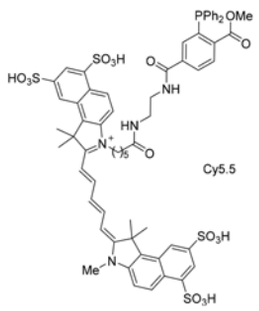
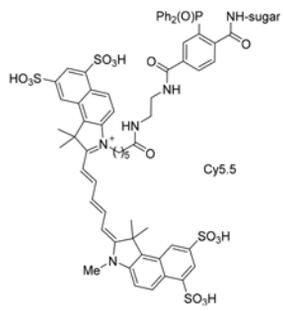
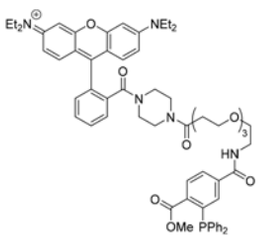
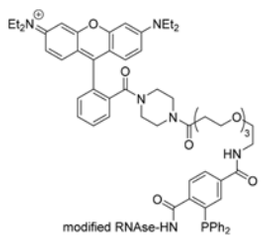
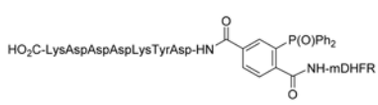
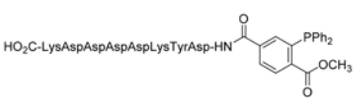
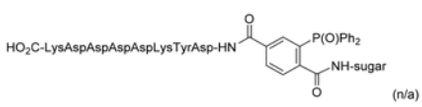
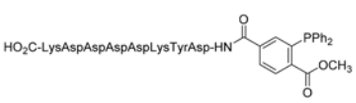
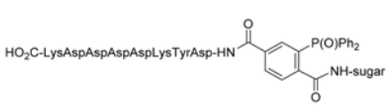
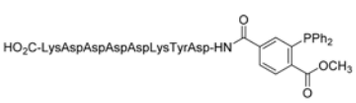
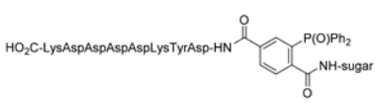
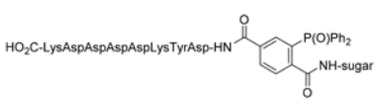
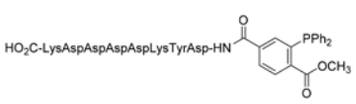
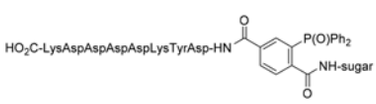
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{63}		Sugar-N_3			217,218
C_{62}		modified RNase	DMSO, PBS buffer, 37 °C, 24 h		226
C_{78}		protein-Cys-S-			163, 197
C_{83}		murine dihydrofolate reductase, mDHFR-N_3		 n/a In cells	227
C_{83}		Different sugar-N_3 (ManNAc and GlcNAc derivatives)		 In cells and *in vivo*	80, 228
C_{83}		Different sugar-N_3 (ManNAz and GlcNAz derivatives)		 n/a In cells	192
C_{83}		Different sugar-N_3 (GalNAc and GlcNAc derivatives)		 n/a In cells	229
C_{83}		Different sugar-N_3 (ManNAc and GlcNAc derivatives)		 n/a In cells	10
C_{83}		Different sugar-N_3 (ManNAc and GlcNAc derivatives)		 n/a	230

Table 1. continued

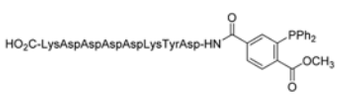
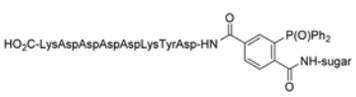
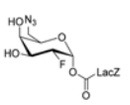
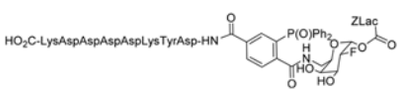
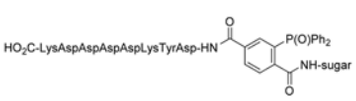
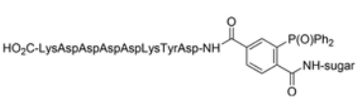
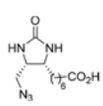
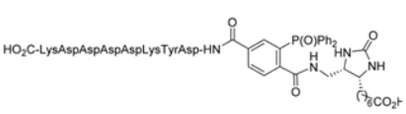
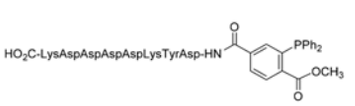
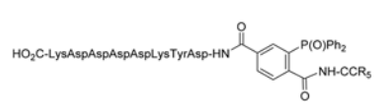
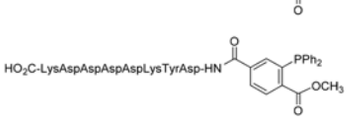
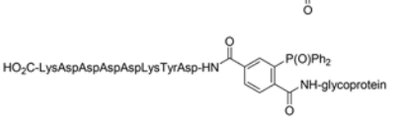
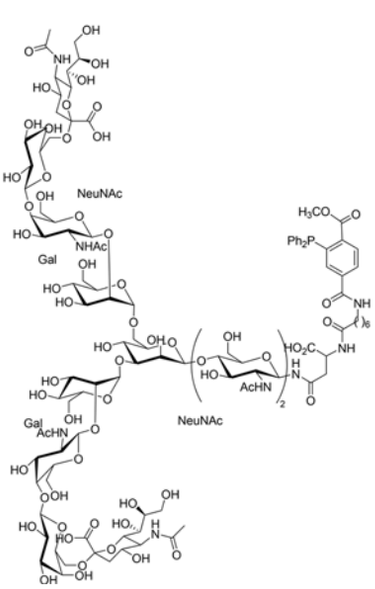
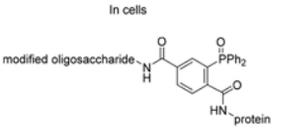
Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
			In cells	
C_{83} 	Different sugar-N_3 (ManNAc and GlcNAc derivatives)			231
			n/a	
			In cells	
C_{83} 	 LacZ: Escherichia coli ß-galactosidase			232
			n/a	
C_{83} 	Different sugar-N_3 (ManNAc and GlcNAc derivatives)			233
			n/a	
			In cells	
C_{83} 	Different sugar-N_3			234
			n/a	
			In cells	
C_{83} 				235
C_{83} 	CCR5-N_3 (C-C chemokine receptor 5)			236, 236
C_{83} 	Glycoprotein-N_3			237
C_{98} 	Different sugar-N_3			217
			n/a	
			In cells	
C_{109} 	Modified protein	n/a		189

Table 1. continued

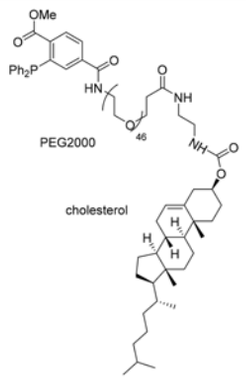
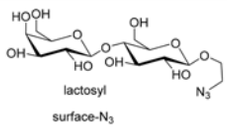
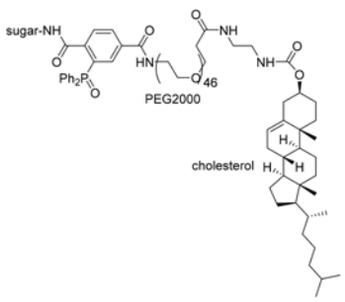
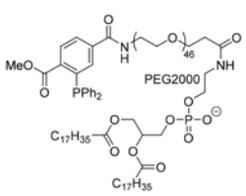
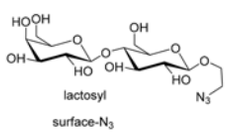
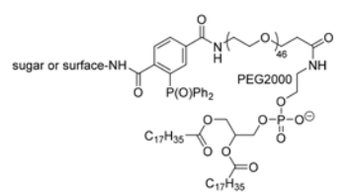
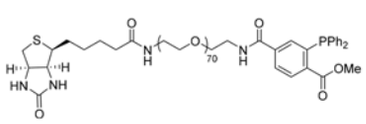
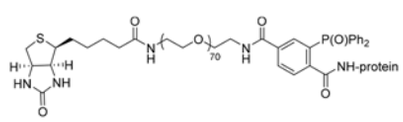
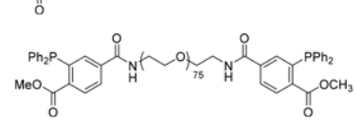
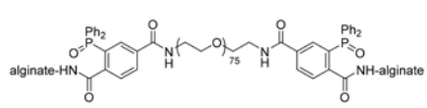
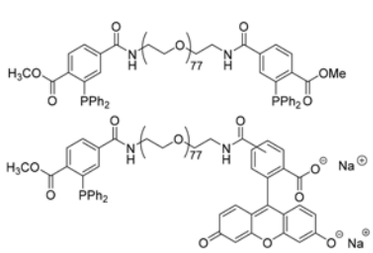
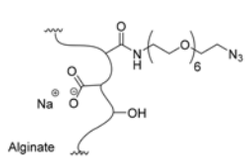
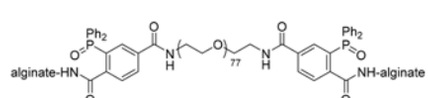
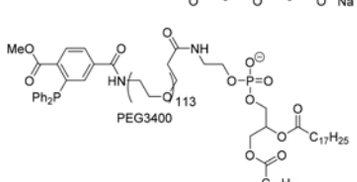
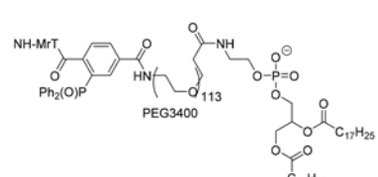
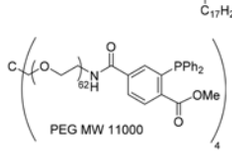
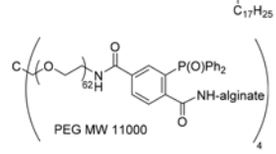
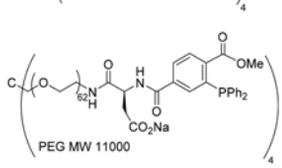
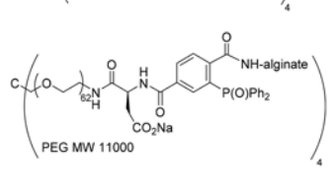
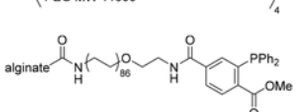
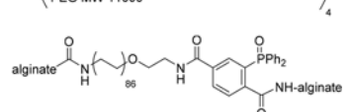
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{148}					238
C_{157}					239,240, 241
C_{178}		Protein-N_3			242
C_{194}		Alginate-N_3			243
C_{198}					244
C_{391}		rTM_{456}-N_3			5
C_{585}		Alginate-N_3			243
C_{601}		Alginate-N_3			243
C_n		Alginate-N_3			243

Table 1. continued

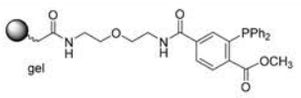
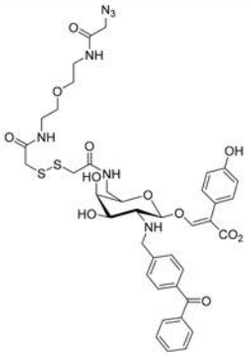
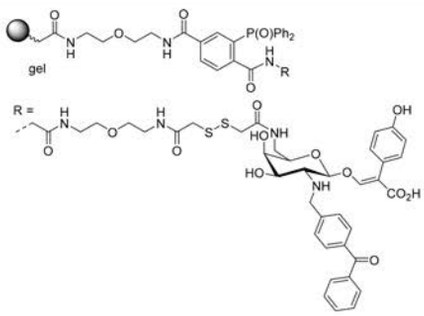
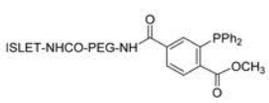
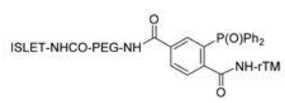
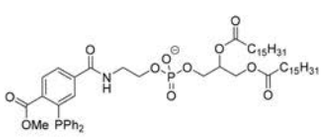
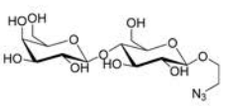
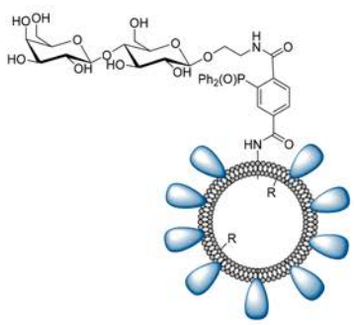
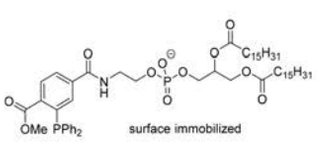
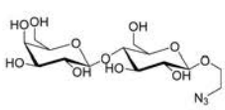
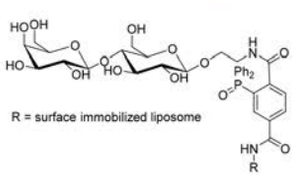
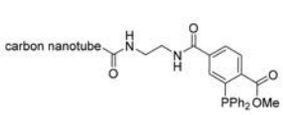
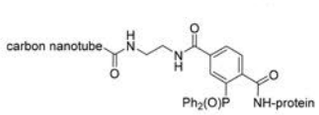
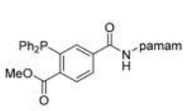
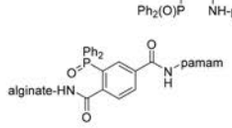
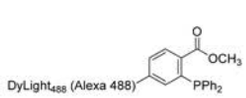
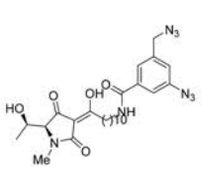
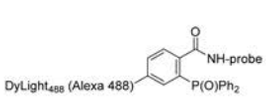
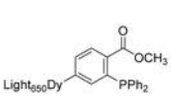
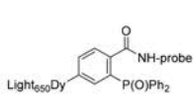
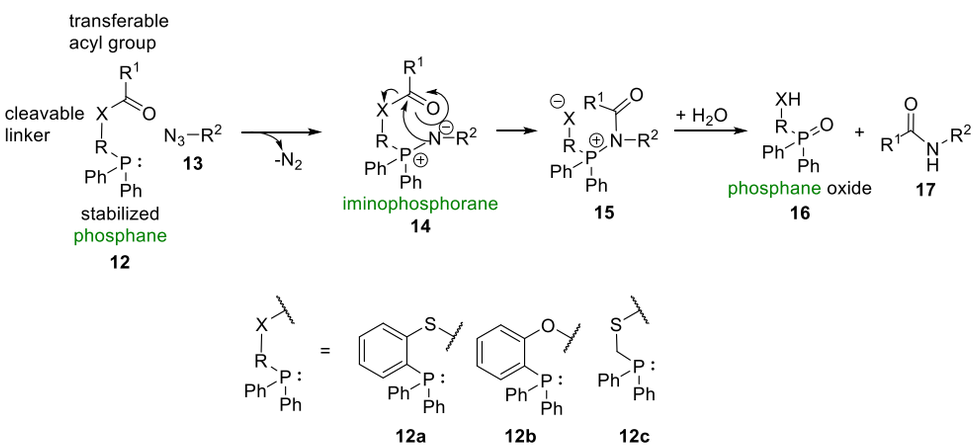
	Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_n					151
C_n		rTM-N_3			245,181
C_n					246
C_n					241
C_n		protein-N_3			247
C_n		Alginate-N_3			248
C_n					249
C_n		Protein with p-azido-L-phenylalanine			250

Table 1. continued

Phosphane	Azides R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_n		*in vivo*		242,148

Scheme 3. Traceless Staudinger Ligation



2.2. Traceless Staudinger Ligation

A related method is the traceless Staudinger Ligation, developed shortly after the introduction of the nontraceless variant, which is used for various applications.[96,97] Vilarrasa and his group demonstrated already in the 1980s, the reaction of carboxylic acids, azides, and phosphanes, yielding amides.[251−253] On the basis of this important preparatory work, the groups of Bertozzi[254] and Raines[255−257] reported in 2000 simultaneously the traceless Staudinger Ligation. This variant is characterized by the formation of an amide bond with concomitant loss of the phosphorus containing moiety **16**. Scheme 3 shows an example for a traceless Staudinger Ligation, starting from an acylated phosphane **12**, which is in most cases a thioester.[254,255] Compound **12** reacts via nucleophilic attack onto the azide **13** to the iminophosphorane **14**, which subsequently traps the negatively charged nitrogen atom via its carbonyl group. Finally, amide **17** and phosphane oxide **16** are formed through hydrolysis (Table 2). The mechanism and kinetics have been corroborated by Raines et al. and Fang et al. in seminal papers.[258−260]

Special features and advantages of the traceless variant include high chemoselectivity in the reaction of phosphane with the azide and a very fast subsequent intramolecular acylation (Table 3). Furthermore, the reaction lacks the requirement for toxic reagents. Currently, the reaction is considered to be one of the most appropriate reactions for the transformation of bio molecules in living cells as it forms naturally occurring peptide bonds as the sole remainders in the product.[7,9]

2.3. Staudinger Phosphite Reaction: A Chemoselective Access to Phosphoramidates

A latter type of the Staudinger Ligation was developed in the 1950s, the Staudinger phosphite reaction, where a phosphite **18** replaces the trivalent phosphane species originally used,[306−309] resulting in loss of one equivalent of nitrogen after reaction with the azide **19** (Scheme 4) and formation of the corresponding trialkyl phosphorimidate **20**. Further elimination of the oxygen bound residue via *P*−*O* cleavage yields a phosphoramidate **21**. The advantages of this variant are (1) easy accessibility of symmetric phosphites, (2) mild aqueous conditions, and (3) simple purification of the products. Moreover, the reaction was recently extended to the use of unsymmetrical phosphites for modification of aryl azides.[310] The Staudinger phosphite reaction is successfully and widely used for chemoselective labeling of proteins or peptides, i.e., by Hackenberger and co workers.[310,311]

3. SCOPE AND LIMITATIONS

3.1. The Azide

In general, aliphatic and aromatic azides, both accessible via various routes,[313] can be used for the Staudinger Ligation (Table 4). However, in the case of aromatic and heteroaromatic azides, the corresponding imidates are formed (Table 5). The azide can also be replaced by other nitrogen electrophiles such as HNO (nitroxyl)[314,315] and nitrosothiols (Table 7).[316]

It should be noted at this point that handling of inorganic azides and small and oligo organic azides needs special precautions as they might react violently under external heating (>150 °C and or shock). Please see refs 317,318 for more information.

Table 2. Traceless Staudinger Ligation[a][110,135,142,144,152,214,239,254,258,260−302]

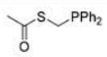
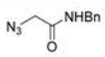
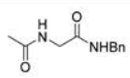
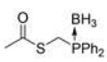
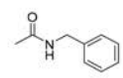
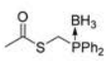
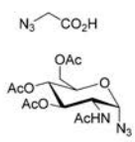
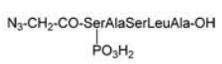
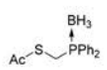
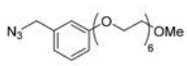
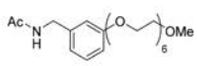
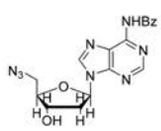
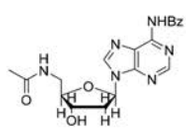
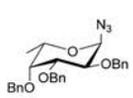
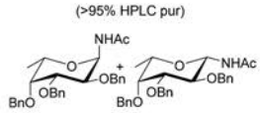
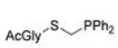
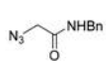
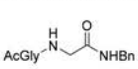
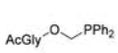
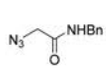
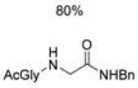
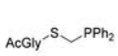
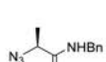
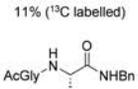
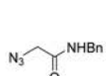
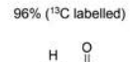
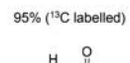
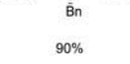
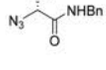
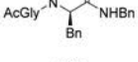
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{15}			3:1 THF/H_2O, 12 h	 91%	260
C_{15}			THF, 1 h, rt; then DABCO, 70 °C, DMF-d^7, 25 °C	 (95% conv)	261
C_{15}		 	DABCO or DIPEA, 40 °C, DMSO-d_6 or DMF-d_7	 Up to 95%	262
C_{15}			TFA, rt, 1 h, then 40 °C, 20 h		263
C_{17}			wet THF, $t_{1/2}$ = 25 h	 (>95% HPLC pur)	254
C_{17}			Various conditions	 up to 70%; from 10:90 to 50:50	264
C_{17}			6:1 DMF/D_2O, 12 h	 80%	258
C_{17}			6:1 DMF/D_2O, 12 h	 11% (^{13}C labelled)	258
C_{17}			6:1 DMF/D_2O	 96% (^{13}C labelled)	258
C_{17}			6:1 DMF/H_2O	 95% (^{13}C labelled)	258
C_{17}			3:1 THF/H_2O, 12 h	 90%	265
C_{17}			3:1 THF/H_2O, 12 h	 93%	265
C_{17}			3:1 THF/H_2O, 12 h	 91%	265

Table 2. continued

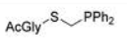
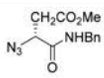
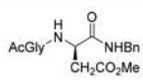
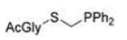
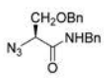
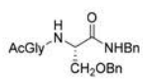
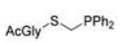
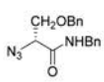
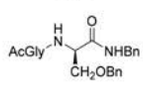
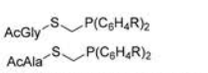
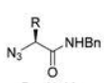
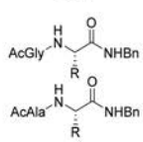
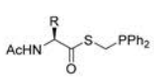
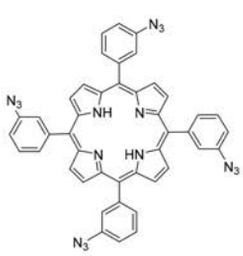
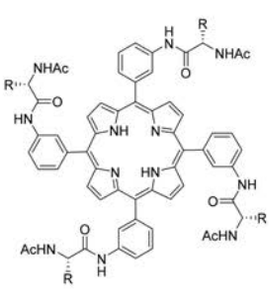
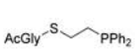
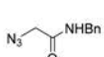
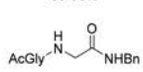
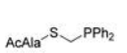
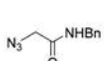
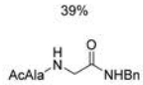
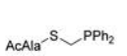
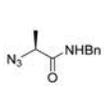
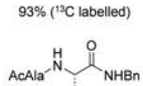
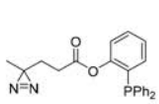
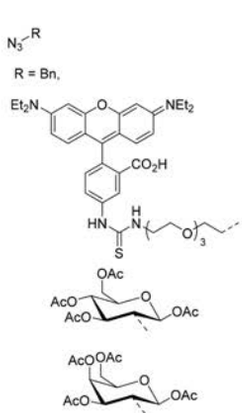
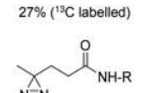
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{17}			3:1 THF/H_2O, 12 h	 95%	265
C_{17}			3:1 THF/H_2O, 12 h	 92%	265
C_{17}			3:1 THF/H_2O, 12 h	 99%	265
C_{17} C_{18} C_{19} C_{20} C_{21} C_{22} C_{23} C_{24}	 R = Cl, H, Me, OMe; OiPr, NMe_2 $C_2H_4NMe_2$, CH_2NMe_2	 R = H, Me	0.4 M sodium phosphite buffer	 n/a (kinetic study, ^{13}C labelled)	256
C_{17} C_{18} C_{24}	 R = H, Me, Bn		DMF/THF or NMP, 50 °C	 89-90%	266
C_{18}			6:1 DMF/D_2O	 39%	258
C_{18}			6:1 DMF/D_2O	 93% (^{13}C labelled)	258
C_{18}			6:1 DMF/D_2O	 27% (^{13}C labelled)	258
C_{23}		 R = Bn,	3:1 THF/H_2O, 12 h	 70-76%	267

Table 2. continued

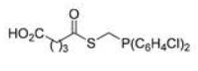
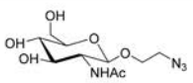
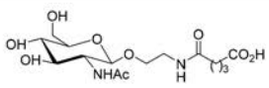
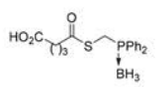
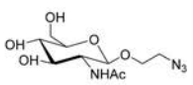
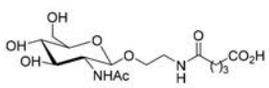
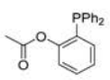
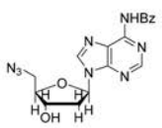
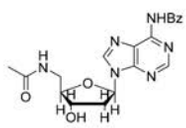
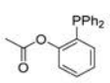
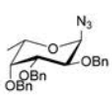
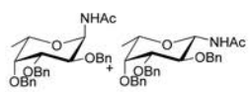
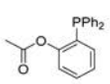
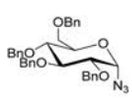
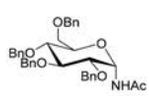
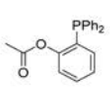
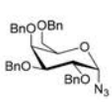
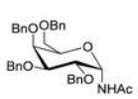
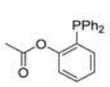
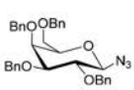
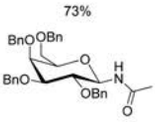
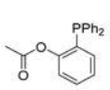
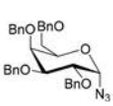
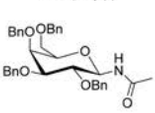
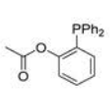
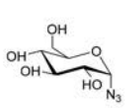
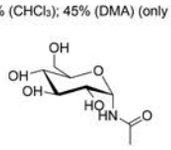
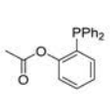
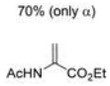
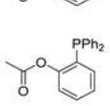
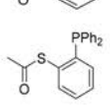
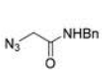
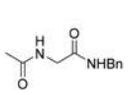
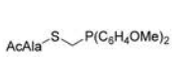
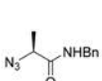
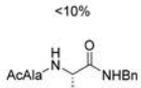
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{18}			5:1 MeCN/H_2O	 37%	268
C_{18}			5:1 MeCN/H_2O	 >95%	268
C_{20}			wet THF, $t_{1/2}$, 18 h	 >95% (HPLC pur)	254
C_{20}			Various conditions	 up to 84%; from 75:25 to 94:6	264
C_{20}			CCl_4, 70 °C, 24 h	 77%	264
C_{20}			CCl_4, 70 °C, 24 h	 73%	264
C_{20}			$CHCl_3$, 70 °C, 24 h	 81% (only β)	269
C_{20}			70 °C, 22 h	 70% ($CHCl_3$); 45% (DMA) (only β)	269
C_{20}			DMA, 70 °C, 18 h	 70% (only α)	270
C_{20}			THF, H_2O	 88%	271
C_{20}		Various azides and other nucleophiles		RNH-Ac	264,272-274
C_{20}			3:1 THF/H_2O, 12 h	 <10%	260
C_{20}			6:1 DMF/D_2O	 81% (^{13}C labelled)	258

Table 2. continued

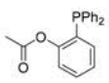
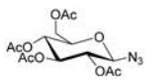
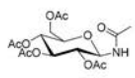
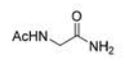
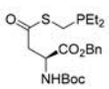
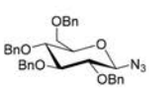
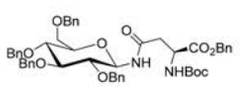
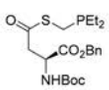
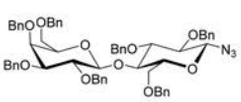
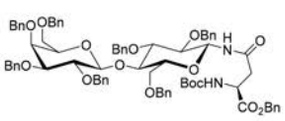
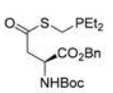
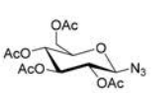
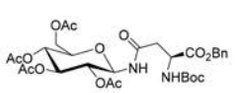
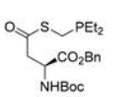
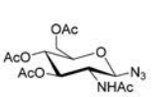
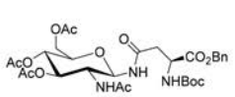
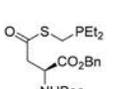
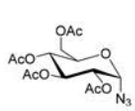
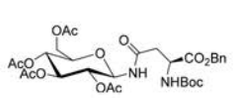
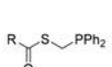
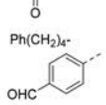
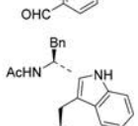
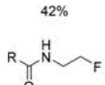
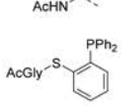
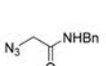
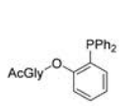
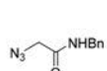
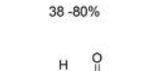
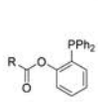
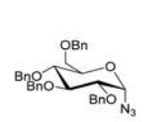
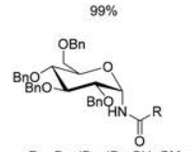
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{20}				 81%	274
C_{21}			0.4 M sodium phosphate buffer	 Ca. 40% (^{13}C labelled)	275
C_{21}			DMF, rt	 54%	276
C_{21}			DMF, rt	 51%	276
C_{21}			DMF, rt	 43%	276
C_{21}			DMF, rt	 32%	276
C_{21}			DMF, rt	 42%	276
C_{21} C_{24} C_{25}	R =   		3:1 THF/H_2O, rt or 80 °C	 61-99%	277
C_{22}			3:1 THF/H_2O, 12 h or 6:1 DMF/D_2O	 38 -80%	260, 258
C_{22}			6:1 DMF/D_2O	 99%	258
C_{23}	 R = Bu, iBu, iPr, CH=CHMe$_2$		$CHCl_3$, 70 °C, 1 h, then irradiation with sunlight 1 h, 70 °C	 R = Bu, iBu, iPr, CH=CMe$_2$ 40-70%	269

Table 2. continued

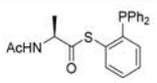
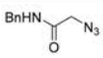
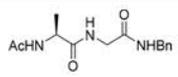
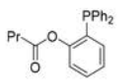
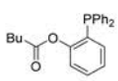
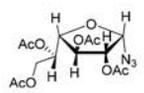
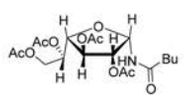
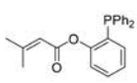
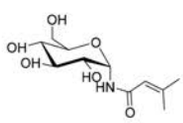
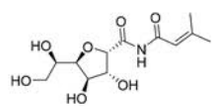
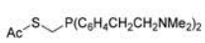
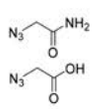
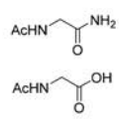
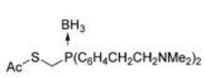
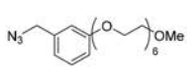
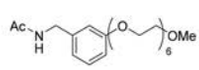
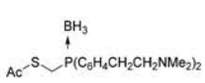
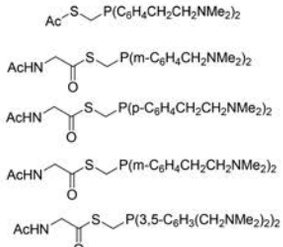
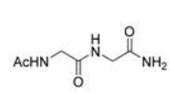
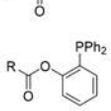
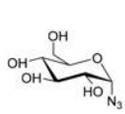
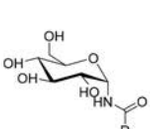
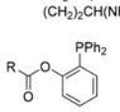
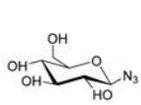
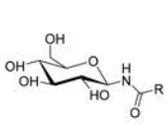
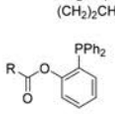
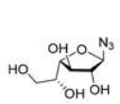
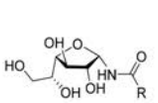
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{22}			3:1 THF/H_2O, 12 h	 35%	255
C_{23}		Various probes		R-NH-COPr	278
C_{23}					270
C_{23}			DMA, microwave, 18 h	 52% (only α); also pyranose	224,270,271
C_{23}			0.4 M sodium phosphate buffer	 >90% (NH_2), >20% (OH) (^{13}C labelled)	275
C_{23}			40 °C, 20 h		263
C_{23}		Fmoc-Nle(w-N_3)AlaGluSerGly-OH	40 °C, 20 h	Fmoc-Lys(Ac)AlaGluSerGly-OH	263
C_{23} C_{25} C_{29}			0.4 M sodium phosphate buffer	 >40% (^{13}C labelled)	256
C_{23} C_{25} C_{31} C_{32}	 R = nBu iBu $(CH_2)_3CO_2CO_2Me$ $CH_2CH(NHCbz)CO_2Me$ $(CH_2)_2CH(NHCbz)CO_2Me$		DMA/DMPU, 40-50 °C, 10 min – 5 h	 35-70% (only α) plus by-products	270
C_{23} C_{25} C_{31} C_{32}	 R = nBu iBu $(CH_2)_3CO_2CO_2Me$ $CH_2CH(NHCbz)CO_2Me$ $(CH_2)_2CH(NHCbz)CO_2Me$		98:2 DMA–DMPU, 50 min, 50 °C MW	 32-75% (only β) plus by-products	270
C_{23} C_{25} C_{28} C_{34}	 R = *n*Bu *i*Bu $CH{=}CMe_2$ $C_{15}H_{31}$ $CH_2CHMe(CH_2)_2CH{=}CMe_2$ Ph		98:2 DMA/DMPU, 70 °C, 4 h or H_2O, 70 °C, 2 h		224

Table 2. continued

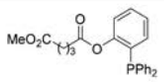
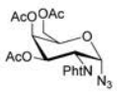
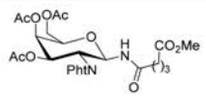
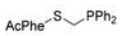
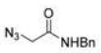
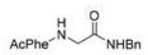
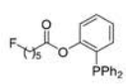
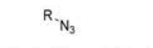
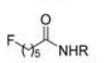
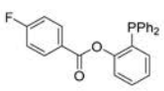
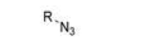
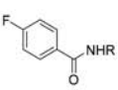
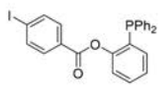
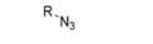
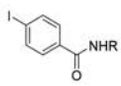
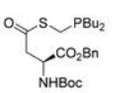
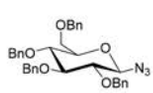
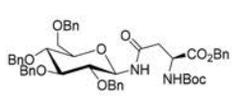
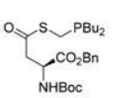
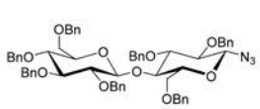
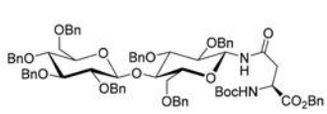
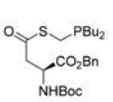
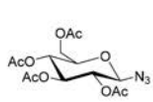
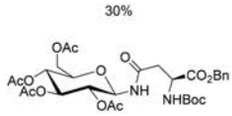
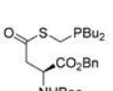
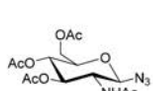
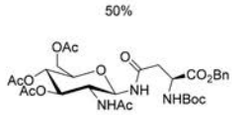
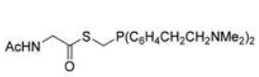
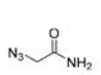
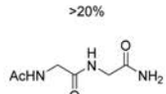
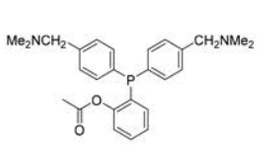
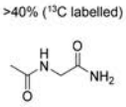
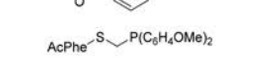
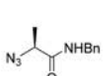
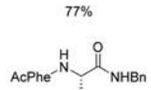
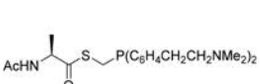
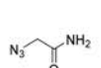
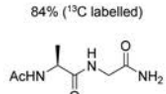
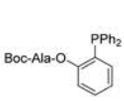
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{24}			70 °C, 22 h	 58% (only β)	269,274,279
C_{24}			3:1 THF/H_2O, 12 h	 92%	260
C_{24}		 R = Bn, Glycosyl, Benzylglycinyl	10:1 DMF/H_2O, 90 °C, 60 min		280
C_{25}		 R = Bn, Glycosyl, Benzylglycinyl	rt or MW	 81-92%	51,281
C_{25}		 R = Bn, Glycosyl, Benzylglycinyl	rt or MW		106,282
C_{25}			DMF, rt	 54%	276
C_{25}			DMF, rt	 30%	276
C_{25}			THF, rt	 50%	276
C_{25}			THF, rt	 >20%	276
C_{25}			0.4 M sodium phosphate buffer	 >40% (^{13}C labelled)	275
C_{26}			D_2O, rt	 77%	283
C_{26}			6:1 DMF/D_2O	 84% (^{13}C labelled)	258,284,285
C_{26}			0.4 M sodium phosphate buffer	 Ca. 60% (^{13}C labelled)	275,286
C_{26}			dioxane, H_2O, 100 °C	74%	271

Table 2. continued

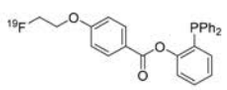
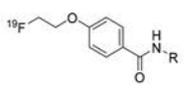
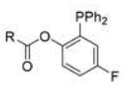
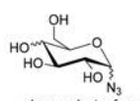
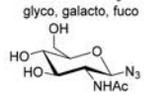
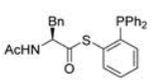
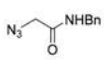
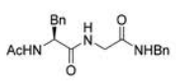
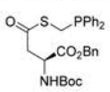
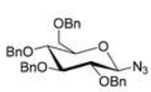
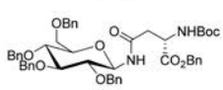
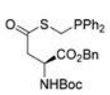
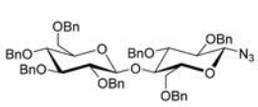
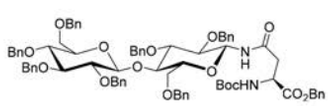
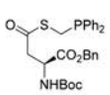
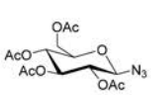
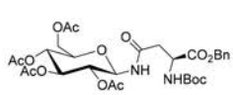
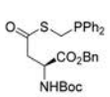
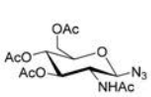
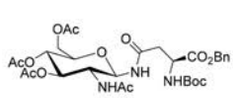
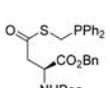
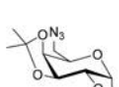
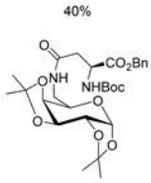
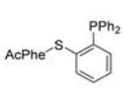
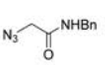
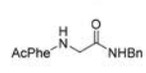
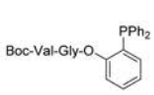
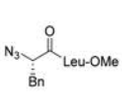
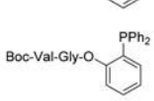
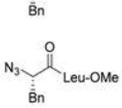
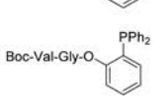
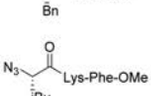
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{27}		 R = Bn, sugar acetonides	10:1 DMF/H_2O, 60 °C	 90-95%	287
C_{28} C_{29} C_{31} C_{32}	 R = $CH_2CH(NHCbz)CO_2Me$ $(CH_2)_2CH(NHCbz)CO_2Me$ $(CH_2)_2NHCbz$ CH_2NHCbz 	 glyco, galacto, fuco 	DMA/DMPU, 16 h, then add H_2O, 2 h, 40 °C		288
C_{29}	 also with glycine		3:1 THF/H_2O, 12-16 h, 0.2 M	 15-35%	255
C_{29}			DMF, rt	 55%	276
C_{29}			DMF, rt	 40%	276
C_{29}			DMF, rt	 40%	276
C_{29}			DMF, rt	 40%	276
C_{29}			toluene, 70 °C, then H_2O	 22-47%	289
C_{29}			3:1 THF/H_2O, rt, 12 h	 35%	260
C_{30}			THF, 47 °C, 16 h, then H_2O, 2 h	Boc-Val-Gly-Phe-Leu-OMe 36%	290
C_{30}			THF, 47 °C, 16 h, then H_2O, 2 h	Val-Gly-Ala-Phe-Leu-OMe 32%	290
C_{30}			THF, 47 °C, 16 h, then H_2O, 2 h	Boc-Val-Gly-Leu-Lys-Phe-NH_2 6%	290

Table 2. continued

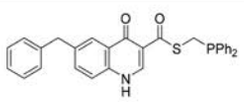
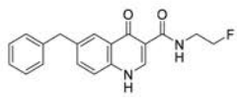
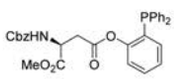
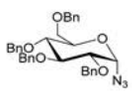
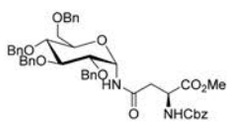
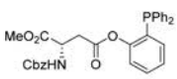
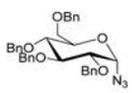
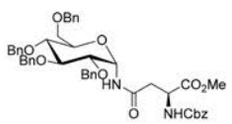
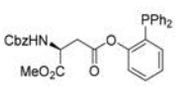
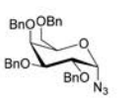
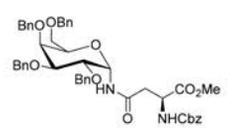
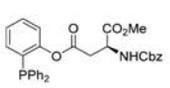
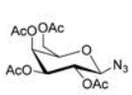
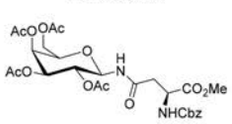
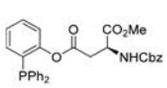
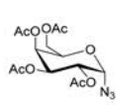
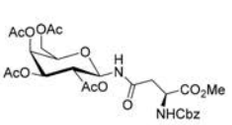
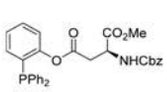
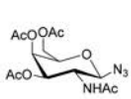
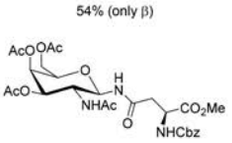
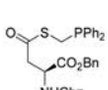
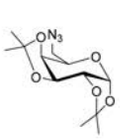
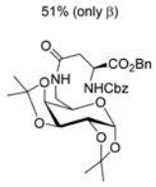
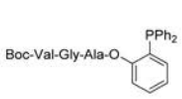
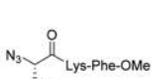
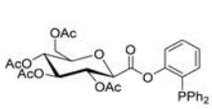
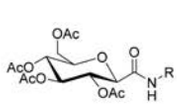
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{30}			DMF/MeCN, 15 min, 130 °C	 n/a	291
C_{31}			1) toluene, 70 °C, 1 h, 2) $CHCl_3$, rt, overnight	 75% (86/14 α:β)	269
C_{31}			Various conditions	 up to 84%, 22:78 to 86:14 α:β	274
C_{31}			1:3 DMA/toluene, 70 °C, 4 h	 65% (65/35 α:β)	110,274,26
C_{31}			DMA, 70 °C, 24 h	 69% (only β)	110,274,26
C_{31}			DMA, 70 °C, 24 h	 54% (only β)	110,274,269
C_{31}			DMA, 70 °C, 24 h	 51% (only β)	110,274,269
C_{32}			toluene, 70 °C, then H_2O	 22-47%	289
C_{33}			THF, 47 °C, 16 h; then H_2O	Boc-Val-Gly-Ala-Leu-Lys-Phe-NH_2 n/d	290
C_{33}		 R = Bn, homoalanines and proteins	DMF/H_2O		292

Table 2. continued

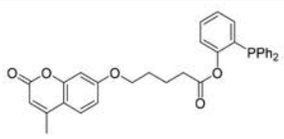
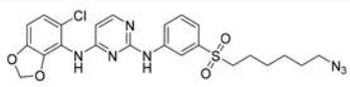
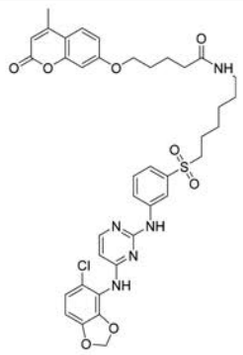
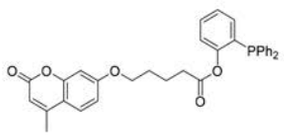
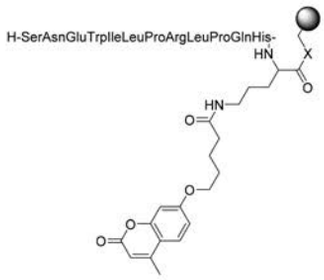
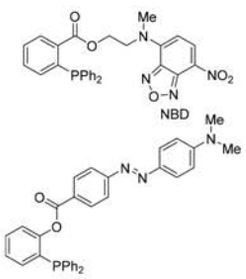
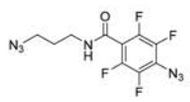
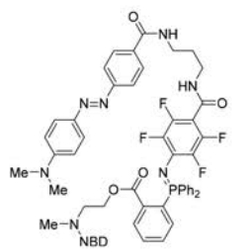
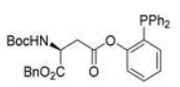
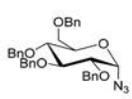
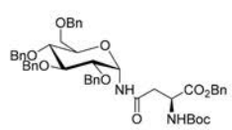
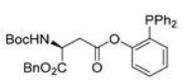
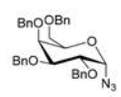
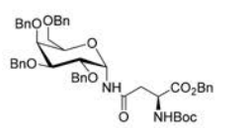
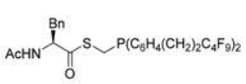
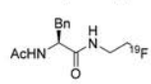
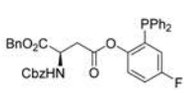
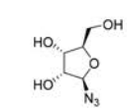
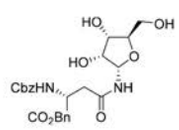
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{33}			10:1 DMF/H_2O, 3 h, 60 °C	 up to 95%	135
C_{33}			10:1 DMF/H_2O, 3 h, 60 °C	 up to 95%	135
C_{33}			One-pot nonhydrolysis Staudinger reaction, 1:4 H_2O-DMF, rt, 96 h		293,142,144
			1:3 DMA/toluene, 70 °C, 4 h	 63% (75/25 α:β)	274,269
C_{34}			1:3 DMA/toluene, 70 °C, 4 h	 71% (68/32 α:β)	274 269
C_{36}			3:1 THF/H_2O, rt, 16 h	 27%	277
C_{37}			98:2 DMA/DMPU, 70 °C, 20 h; then H_2O, 2 h, 70°C	 Plus pyranosyl	294

Table 2. continued

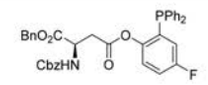
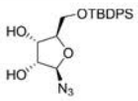
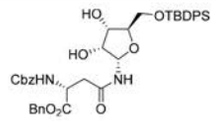
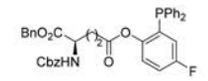
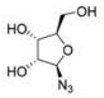
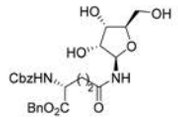
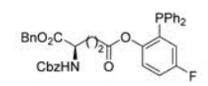
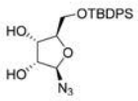
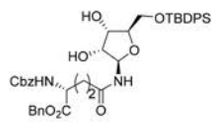
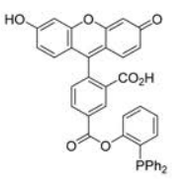
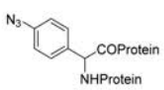
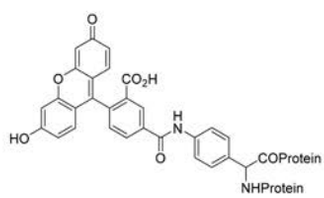
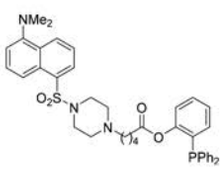
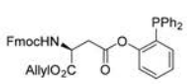
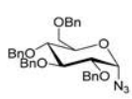
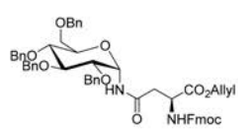
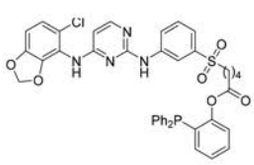
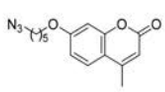
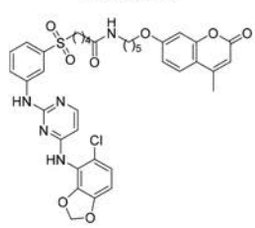
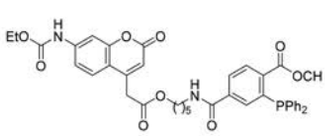
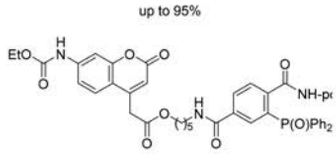
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{37}			DMA/DMPU, 70 °C, 20 h	 60%	294
C_{38}			DMA/DMPU, 70 °C, 20 h	 Plus pyranosyl	294
C_{38}			DMA/DMPU, 70 °C, 20 h	 76%	294
C_{39}			PBS, pH 7.4, rt, 16 h On a phage		214
C_{39}		Cetuximab$(N_3)_{25}$ (monoclonal antibody)	PBS, pH 7.4, 37 °C, 6 h	Cetuximab-$(N_3)_{14}$-(dansylpiperazinyl)$_{11}$	295
C_{40}			1:3 DMA/toluene, 70 °C, 4 h	 65% (70/30 α:β)	274,269
C_{40}			10:1 DMF/H_2O, 3 h, 60 °C	 up to 95%	135
C_{40}		polymer-N_3		 n/a	296
C_{49}	Boc-Ser(β-Gal(OAc)$_4$)-Phe-Gly-Ala-SCH_2PPh_2	N_3-CH_2-CO-Phe-Val-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Ser(β-Gal(OAc)$_4$)-Phe-Gly-Ala-Gly-Phe-Val-Ala-Leu-OH 61%	152

Table 2. continued

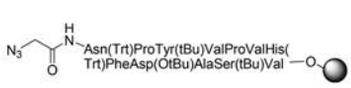
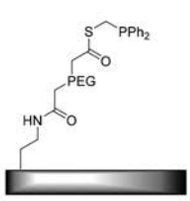
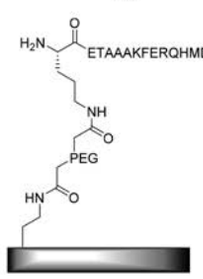
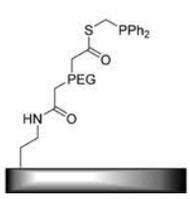
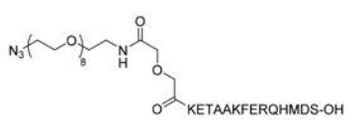
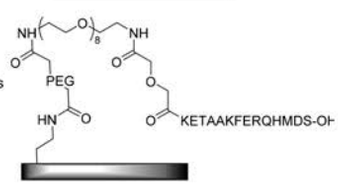
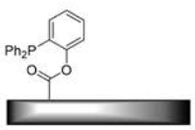
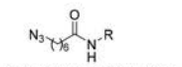
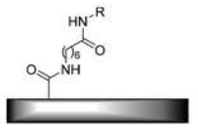
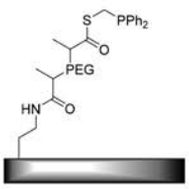
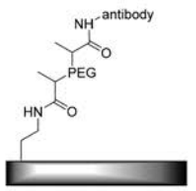
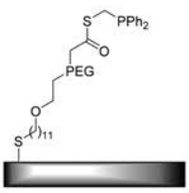
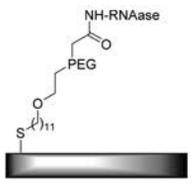
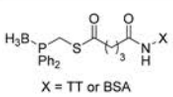
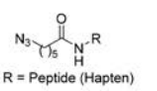
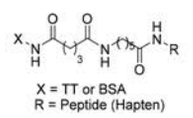
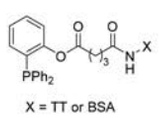
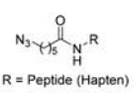
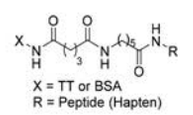
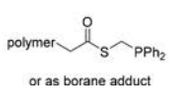
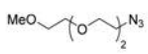
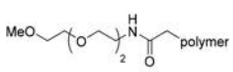
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{49}	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-SCH_2PPh_2	N_3-CH_2-CO-Phe-Val-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-Gly-Phe-Val-Ala-Leu-OH 68%	152
C_{49}	Boc-Ser(β-Gal(OAc)$_4$)-Phe-Gly-Ala-SCH_2PPh_2	N_3-CH_2-CO-Ser-Val-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Ser(β-Gal(OAc)$_4$)-Phe-Gly-Ala-Gly-Ser-Val-Ala-Leu-OH 66%	152
C_{49}	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-SCH_2PPh_2	N_3-CH_2-CO-Ser-Val-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-Gly-Ser-Val-Ala-Leu-OH 64%	152
C_{49}	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-SCH_2PPh_2	N_3-CH_2-CO-Phe-Ala-Ser(β-Gal(OAc)$_4$)-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-Gly-Phe-Ala-Ser(β-Gal(OAc)$_4$)-Leu-OH 64%	152
C_{49}	Boc-Phe-Ser-(β-Gal(OAc)$_4$)- Gly-Ala-SCH_2PPh_2	N_3-CH_2-CO-Pro-Phe-Asn(β-Gal(OAc)$_4$)-Ala-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-Gly-Phe-Asn(β-Gal(OAc)$_4$)-Ala-OH 70%	152
C_{53}	H-Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro-SCH_2PPh_2	$(N_3CH_2COOCO)_2$-[DMEDA-PEG-DMEDA-(MBA-DMEDA)$_{n+1}$-PEG-DMEDA]	PBS, pH 7	(Arg-Gly-Asp-Ser-Pro-Ala-Ser-Ser-Lys-Pro-$NHCH_2COOCO)_2$-[DMEDA-PEG-DMEDA-(MBA-DMEDA)$_{n+1}$-PEG-DMEDA]	297
C_{57}	Boc-Phe-Gly-Val-Ala-SCH_2PPh_2	N_3-CH_2-CO-Phe-Val-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Gly-Val-Ala-Gly-Phe-Val-Ala-Leu-OH 67%	152
C_{57}	Boc-Phe-Gly-Val-Ala-SCH_2PPh_2	N_3-CH_2-CO-Ser(β-Gal(OAC)$_4$)-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Gly-Val-Ala-Gly-Ser(β-Gal(OAc)$_4$)-Phe-Ala-Leu-OH 31%	152
C_{57}	Boc-Phe-Gly-Val-Ala-SCH_2PPh_2	N_3-CH_2-CO-Phe-Ser(β-Gal(OAc)$_4$)-Ala-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Gly-Val-Ala-Gly-Phe-Ser(β-Gal(OAc)$_4$)-Ala-Leu-OH 63%	152
C_{57}	Boc-Phe-Gly-Val-Ala-SCH_2PPh_2	N_3-CH_2-CO-Phe-Ala-Ser(β-Gal(OAc)$_4$)-Leu-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Gly-Val-Ala-Gly-Phe-Ala-Ser(β-Gal(OAc)$_4$)-Leu-OH 70%	152
C_{59}	Fmoc-Cys(Trt)-Glu(O^{t}Bu)-SCH_2PPh_2		10:1 DMF/H_2O, 12 h, rt Resin bound	FmocCys(Trt)Glu(OtBu)Asn(Trt)ProTyr(tBu)ValProValHis(Trt)PheAsp(OtBu)AlaSer(tBu)Val, RNase A(110–124)	298
C_{65}	Fmoc-Gly-Asn(β-Gal(OAc)$_4$)-Ala-Pro-Phe-SCH_2PPh_2	N_3-CH_2-CO-Phe-Ala-Ser(β-Gal(OAc)$_4$)-Ala-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-Gly-Phe-Ala-Ser(β-Gal(OAc)$_4$)-Ala-OH 64%	152
C_{65}	Fmoc-Gly-Asn(β-Gal(OAc)$_4$)-Ala-Pro-Phe-SCH_2PPh_2	N_3-CH_2-CO-Pro-Phe-Asn(β-Gal(OAc)$_4$)-Ala-OH	2:1 DMF/H_2O, rt, 72 h	Boc-Phe-Ser(β-Gal(OAc)$_4$)-Gly-Ala-Gly-Pro-Phe-Asn(β-Gal(OAc)$_4$)-Ala-OH 64%	152
C_n			Sodium phosphate buffer Phosphane on a glass slide		298
C_n			Sodium phosphate buffer Phosphane on a glass slide		298

Table 2. continued

Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
	 R = biotinyl, mannosyl, biaryl	Phosphane on a glass slide		298
	Antibody-N_3	50:1 DMF/H_2O, 4 °C, 2.5 h Phosphane on a glass slide		299
	Azido-RNAase	Sodium phosphate buffer Phosphane on a gold slide		300
 X = TT or BSA	 R = Peptide (Hapten)	a) potassium phosphate buffer, 0.1 M, pH 7.4, 47 °C, 6 h; b) DABCO (40 + 40 equiv), 5:1 DMF/NaCl 0.05 M, 40-45 °C, 4 h	 X = TT or BSA R = Peptide (Hapten)	268
 X = TT or BSA	 R = Peptide (Hapten)	Potassium phosphate buffer 0.1 M, pH 7.4, 47 °C, 6 h	 X = TT or BSA R = Peptide (Hapten)	268
 or as borane adduct				301

[a]The traceless Staudinger reaction was also applied to cyclization reactions (Table 3).

3.2. The Phosphane

In general, aromatic and aliphatic phosphanes can be used (for side reactions, see below) for the Staudinger Ligation. Electron rich phosphanes are found to perform with greater reactivity in comparison to electron deficient phosphanes.[258] In some cases, water soluble reagents proved to be superior.[283−289] Moreover, fluorous variants of the phosphane have been shown to enhance separation from the byproducts.[277,278]

In the Staudinger reaction, a leaving group on the carbonyl group is necessary to install the intramolecular electrophile. In most of the cases, methoxide serves this purpose. However, other alkoxides including fluorogenic ones[319] can be used as well.

3.3. Reaction Conditions and Side Reactions

The Staudinger Ligation was designed to provide the desired amide structure in aqueous solutions. As such, in general, water is the common solvent, however, nonpolar solvents gave rise to high yields, which is supported by DFT calculations of the intermediates.[258] In some cases, in particular with anomeric sugar azides, different solvents gave rise to variable diastereomeric ratios.[274] Besides, the pH dependence has been demonstrated in several communications.[150−157,275]

Additives are in general not required for the Staudinger Ligation. It should be mentioned at this point that most aliphatic phosphanes are oxygen sensitive, hence in nonbiological systems exclusion of oxygen is envisaged. This is, of course, not possible in all biological systems such as whole animals.[228] Therefore, side reactions from oxidation of the phosphane are observed. This can be circumvented by using borane protected phosphanes (see Scheme 11).

Another serious side reaction is the premature hydrolysis of the iminophosphorane to yield either phosphorylamines (see Scheme 4) or amines (Staudinger reduction).[320,321] Reactive electrophiles such as Michael acceptors react with thiols released from the traceless Staudinger Ligation of thioesters: this can be circumvented using esters.[271]

3.4. Stereochemistry

In general, stereochemical issues do not play any role in the Staudinger Ligation. However, in certain cases, the stereochemistry of the azide can either be retained or inverted. This has been demonstrated by Bertozzi in a stereoselective Staudinger Ligation to glycoproteins. Here, an activated ester and phosphane were present in the same molecule. This resulted in glycosylamide bond formation through condensation of a glycosylamine and an activated carboxylic acid. However, a high tendency of the glycosylamides to isomerization has the effect that in most cases an anomeric mixture is obtained.[276] Nevertheless, a stereoselective traceless Staudinger Ligation was already reported as well. Here, the protecting groups of the sugar compounds **22** were exploited to generate stereoselectivity

Table 3. Intramolecular Traceless Staudinger Ligation[a][124,135,153,261,262,302−305]

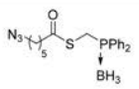
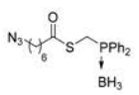
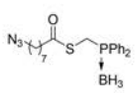
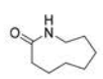
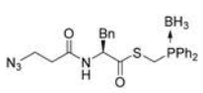
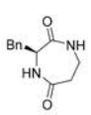
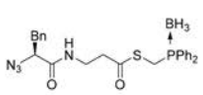
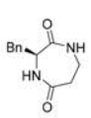
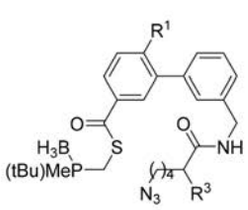
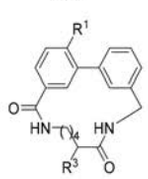
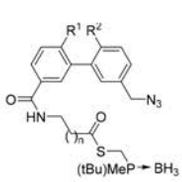
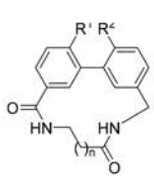
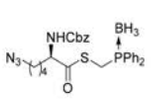
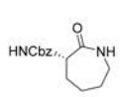
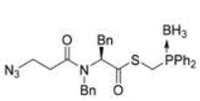
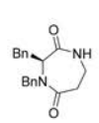
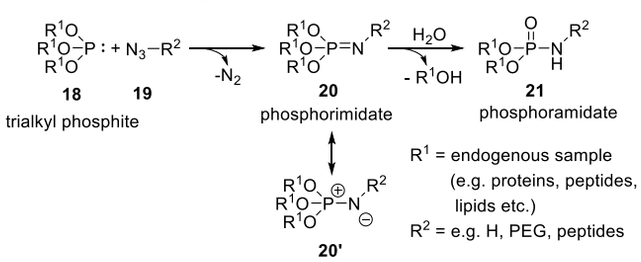
	Starting material	Conditions	Products and Yields	Refs.
C_{19}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 84%	302
C_{20}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 59%	302
C_{21}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 80%	302
C_{25}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 29%	302
C_{25}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 29%	302
C_{26} C_{27} C_{34}	 R^1, R^3 = H, H R^1 = Me, R^3 = H R^1 = Me, R^3 = NHCbz	DABCO, THF, reflux, 18 h	 57-65%	135,124
C_{25} C_{26} C_{27} C_{28}	 R^1, R^2 =H, n = 4 R^1 =Me, R^2 = H, n = 4 R^1, R^2 =H, n = 3 R^1, R^2 = Me, n = 4	DABCO, THF, reflux, 18-48 h	 40-72%	135,124
C_{27}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 80%	302
C_{32}		DABCO, 99.5:0.5 THF/H_2O, 70 °C	 35%	302

Table 3. continued

	Starting material	Conditions	Products and Yields	Refs.
C_{63}		DABCO, 70 °C, THF	$C_{50}H_{71}N_{11}O_{14}$: c-GIGTPISFYGG 20%	261
C_{66}		DABCO, 70 °C, THF	$C_{53}H_{71}N_{13}O_{14}$: c- GAGHVPEYFVG 36%	261
C_{66}		DABCO, 70 °C, THF	$C_{53}H_{71}N_{13}O_{14}$: c-GGIVPQFYSAG (31)	261
C_{66}		TFA/TIS; then DIPEA	c-GlyAlaGlyArgValProGluTyrPheValGly n/a	262
C_{112}		BITFA/TIS; then DIPEA	AlaGlyHisValProGluTyrPheValGlyIleGly ThrProIleSerPheTyrGlyGly n/a	262

[a]In case of aromatic azides, imidates are formed (Table 4 and Table 5).

Scheme 4. Staudinger Phosphite Reaction[306−309]



for the directed synthesis of α or β glycosylamides (**24** or **25**, Scheme 5).[264−282]

A stereoselective Staudinger Ligation was also performed for β *N* glycosylation of peptides (Scheme 6).[276] The reaction starts with an azido sugar **26** as an anomeric mixture, which reacts with phosphinothioester modified asparagines again to an anomeric mixture of the α and β iminophosphorane intermediates **27** and **28**. Modulations of the phosphane substituents cause changes in steric and electronic properties of the iminophosphorane intermediates **30** and **31**, which finally lead to a defined stereochemistry of the β *N* glycan **32**. Starting from the α iminophosphorane compound, the generation of the α *N* glycosylamide **29** is prevented.

Miscellaneous Methods. Apart from the methods described above, other labeling techniques have been used including a Staudinger Ligation as a key step, for example, for drug release.[322]

4. APPLICATIONS TO SYNTHESIS AND BIOLOGY

4.1. Staudinger Ligation for Labeling of Biomolecules

There are many applications of Staudinger Ligations, ranging from total syntheses,[149,271] radiolabeling,[105,323] polymer and material syntheses,[144,324−326] and imaging[27] to (and this is the main application) ligation of biomolecules such as sugars, proteins or peptides.[326]

4.1.1. Biotin Labeling. Biotin's strong affinity to streptavidin makes it an essential tool for biomacromolecule derivatization in vitro and in vivo as streptavidin eventually can be subjected to various modifications, e.g., fluorophore coupling for imaging purposes. The use of Staudinger Ligations for this kind of biotin linkage is reported frequently.[10,116,117,120,162,173194] For instance, it was shown that biotin conjugation enables the labeling and isolation of proteins in vivo.[194−197] Furthermore, biotin labeling can be used to detect proteins modified with fatty acids as demonstrated by Bertozzi and co workers.[10]

In spite of the fact that the Staudinger Ligation is often favored due to its nontoxic reaction conditions, there are still some limitations of Staudinger based methodologies that make the use of other bioconjugation methods essential. For example, in a study by Marnett et al. comparing Staudinger Ligation with an azide−alkyne based click reaction for biotin labeling of proteins in cancer cells a more difficult purification of the proteins after streptavidin coupling was reported.[162]

4.1.2. Fluorophore Labeling. As already depicted in the previous section, the Staudinger Ligation using phosphane−biotin conjugates in vivo is a suitable and frequently selected method for the introduction of bioorthogonal chemical reporters into intracellular biomolecules.[160,319,327] A subsequent conversion with fluorescently labeled streptavidin finally allows visualization of the respective molecule. One overall advantage of this method is that no genetic manipulation is required.[68] However, incomplete removal of unbound phosphane−biotin entities in in vivo or in vitro systems limits the use for some applications as this enhances the background fluorescence.[160] That drawback could be solved by direct application of phosphane fluorophores because this results in specific labeling of targeted molecules. Indeed, this alternative reaction may again lead to an undesirable increase of the background fluorescence. The trigger for this is the formation of phosphane oxide byproducts due to an easy oxidizability of phosphanes.

So far, two new approaches have been developed which proved to be better suited for fluorescent labeling with Staudinger Ligation as background fluorescence is substantially decreased. A first method focuses on the use of nonfluorescent dye precursors like the 7 amino coumarin phosphane dye **33** shown in Scheme 7.[160] Because of the coupling of the precursor **33** to the target molecule via addition of N_3 R, the dye switches from a photophysically inactive phosphane **33** to the phosphane oxide **34** in an active state. The reason for the occurrence of these two states is the electronic influence of the lone pair of electrons of the phosphorus prior to the Staudinger Ligation,

Table 4. Other Staudinger Ligations: Aromatic Azides[303,304]

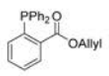
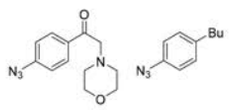
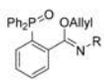
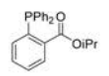
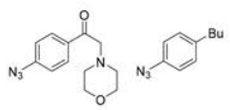
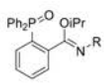
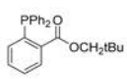
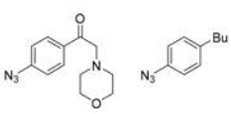
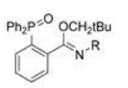
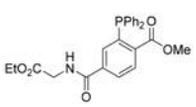
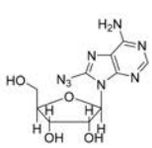
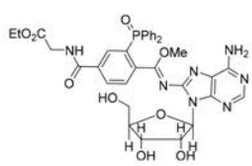
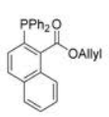
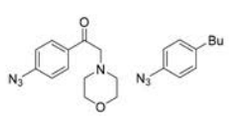
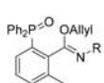
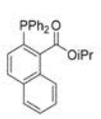
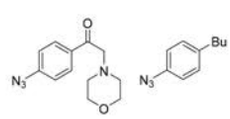
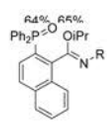
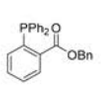
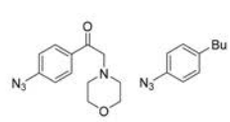
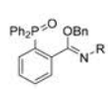
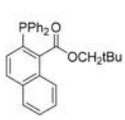
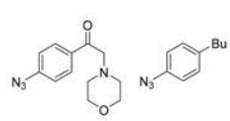
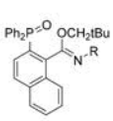
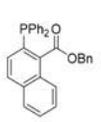
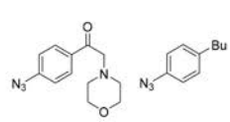
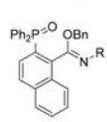
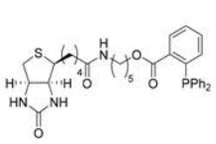
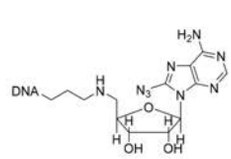
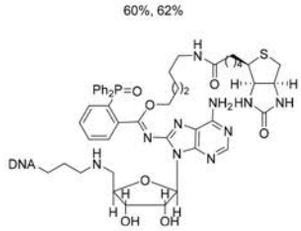
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C_{22}			1:1 THF/H_2O, rt, 1.5 h	 64%, -	303
C_{22}			1:1 THF/H_2O, rt, 1.5 h	 66%, -	303
C_{24}			1:1 THF/H_2O, rt, 1.5 h	 67%, -	303
C_{25}			1:1 THF/H_2O, 30 °C, 2 h	 97%	303
C_{26}			1:1 THF/H_2O, rt, 1.5 h		303
C_{26}			1:1 THF/H_2O, rt, 1.5 h	 40%, 55%	303
C_{26}			1:1 THF/H_2O, rt, 1.5 h	 53%	303
C_{28}			1:1 THF/H_2O, rt, 1.5 h	 38%, 40%	303
C_{30}			1:1 THF/H_2O, rt, 1.5 h	 60%, 62%	303
C_{35}			H_2O, 50 mM, NaOH, 37 °C, 14 h	 n/a	304

Table 4. continued

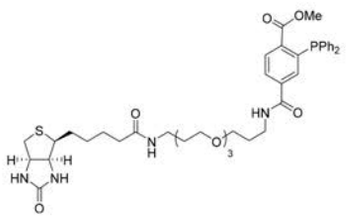
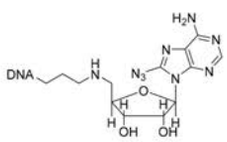
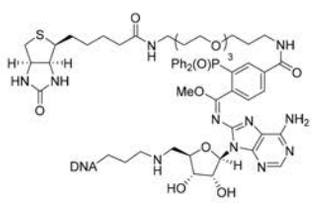
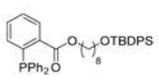
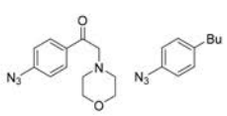
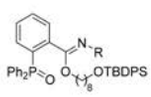
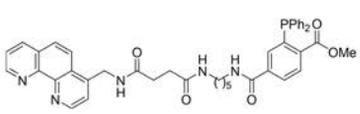
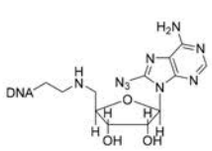
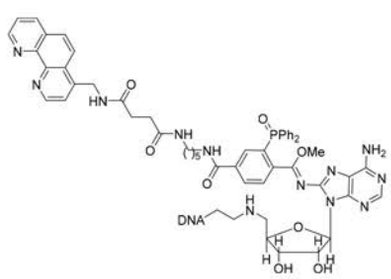
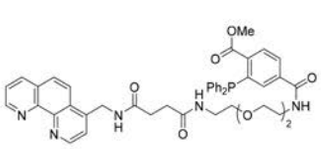
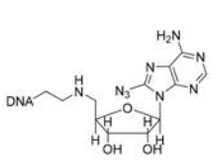
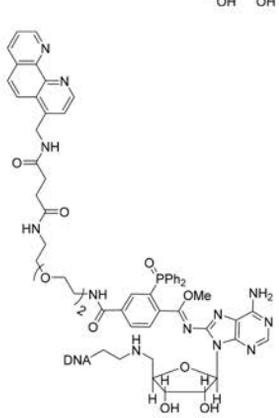
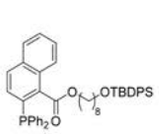
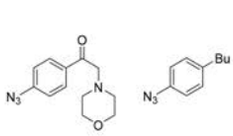
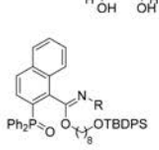
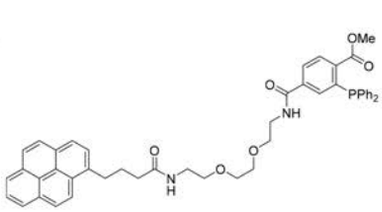
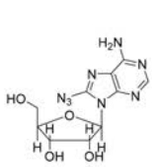
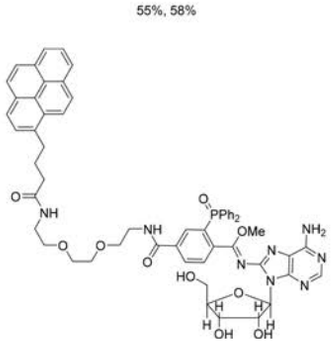
	Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C41			1:1 THF/H_2O	 n/a	304
C43			1:1 THF/H_2O, rt, 1.5 h	 66%, -	303
C44			1:1 THF/H_2O		304
C44			1:1 THF/H_2O		304
C47			1:1 THF/H_2O, rt, 1.5 h	 55%, 58%	303
C47			1:1 THF/H_2O		303

Table 4. continued

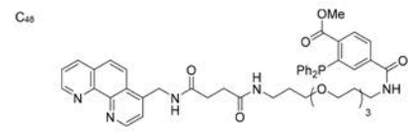
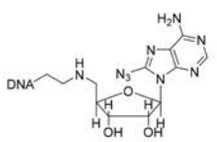
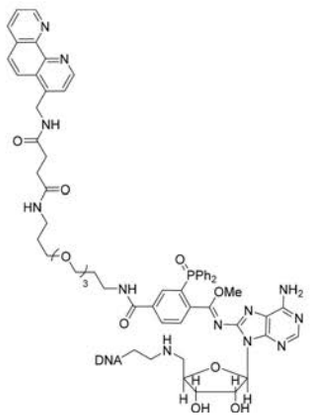
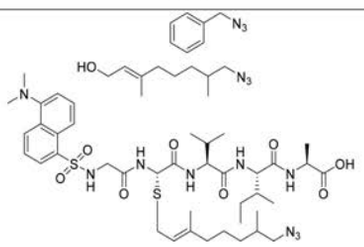
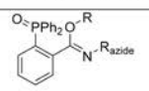
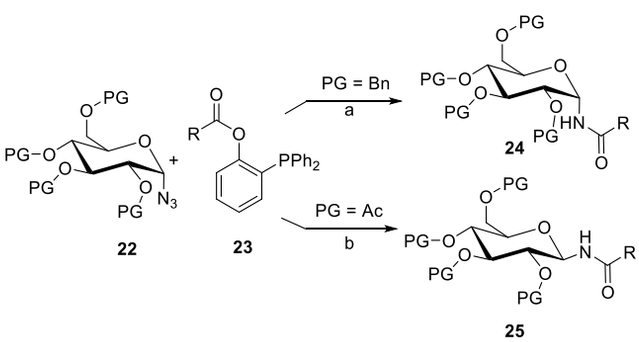
Phosphane	Azide	Conditions	Product(s) and Yields	Refs.
C48 		1:1 THF/H_2O		304

Table 5. Other Staudinger Ligations Leading to Imidates[153,305]

Phosphane	Azide R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C20  C21 R = Me, Et		CD_3CN, rt	 (n/a: mass spectroscopical investigations)	153,305

Scheme 5. Stereoselective Traceless Staudinger Ligation for Synthesis of α or β Glycosylamides



which quenches the excited state until it finally comes to oxidation.

In another experiment, the group used a similar approach, but instead of changing electronic effects an internal quencher was incorporated directly into the molecule (**35** and **35a**, Scheme 8). Because of the replacement of the quencher unit by the azide moiety **37** in the Staudinger Ligation, fluorescence of the target molecule **38** was enabled.[319]

Some fluorescent entities as, e.g., coumarins, naphthylamides, and lately also fluorescein and rhodamine dyes, have been utilized so far. A wide variety of biomolecules have been derivatized, such as DNA, carbohydrates, or even phages.[213,214,215,231,233]

4.1.3. DNA Labeling. The Staudinger Ligation has further been used for labeling of DNA. First, a study already showed the successful labeling of the 5′ terminus of DNA.[213] Furthermore, this reaction was applied for methyltransferase mediated DNA labeling.[304,305] Azide modified triphosphates like **39** were also used to incorporate azide moieties into the DNA backbone (**40**).[191,134,173] Conjugation with modified phosphanes **41** yielded biotin labeled DNA **42**.[190] A brief overview of this application is shown in Scheme 9.

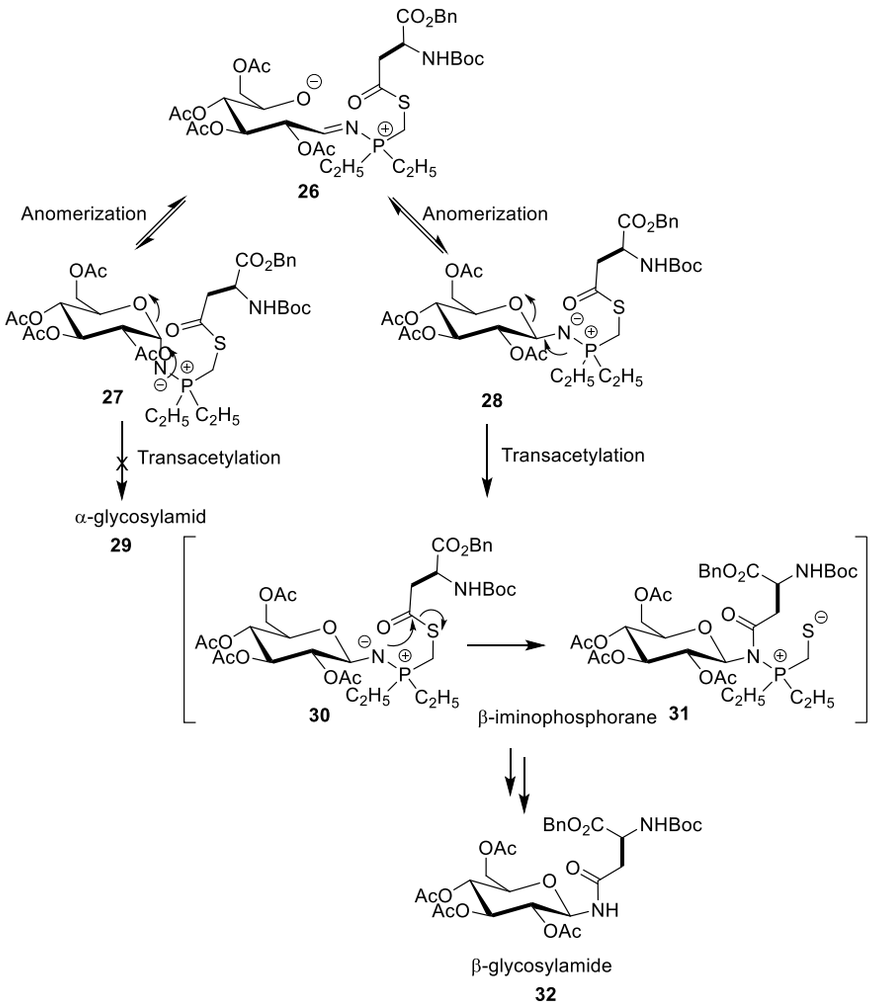
4.1.4. Further Labeling Molecules. Aside from the presented methods for conjugation, several other molecules for biolabeling *via* Staudinger Ligation are known and in use. One alternative method is the labeling of nucleosides using ferrocene or coumarin. In this case, it is important that the reducing reagent and the electrophile are not provided in one chemical entity.[328] Another interesting method is the FLAG tagging introduced by Stubbs and co workers.[127] The aim of their study was the identification of β glucosaminidases in the proteome of *Pseudomonas aeruginosa*.[232,234] Also, the group of Bertozzi applied the FLAG tag method for successful in vivo labeling of glycostructures such as mucin type *O* glycans.[231,233]

4.2. Staudinger Ligation Involving Peptides and Proteins

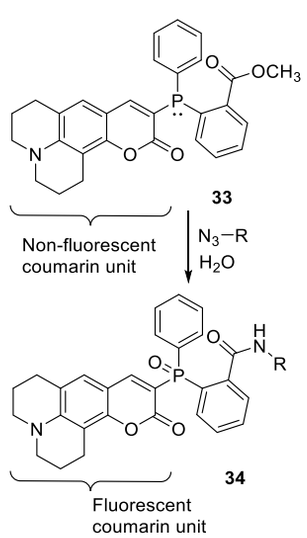
The Staudinger Ligation was also addressed for a couple of reactions with regard to peptides and proteins, i.e., conjugation reactions or various modifications. First, a novel method was developed for synthetic protein assembly, as a disadvantage of current reactions like native chemical ligation or auxiliary mediated native chemical ligation is that for successful coupling a terminal cysteine or glycine residue is necessary. Therefore, as a new feature, other types of amino acids may be present at the ligation site. In this approach, the traceless variant of the Staudinger Ligation is used. Initially, the *N* terminal amino acid is azide functionalized (**44**). It is then attacked by a *C* terminal phosphinothioester **43** of the second peptide (Scheme 10).[1]

There were already some different phosphanes for successful peptide−peptide ligations reported, of which few examples are shown in Figure1.[81,255,258]

Scheme 6. Stereoselective Traceless Staudinger Ligation for *β N* Glycosylation of Peptides



Scheme 7. Coumarin–Phosphane Dye Activated upon Staudinger Ligation



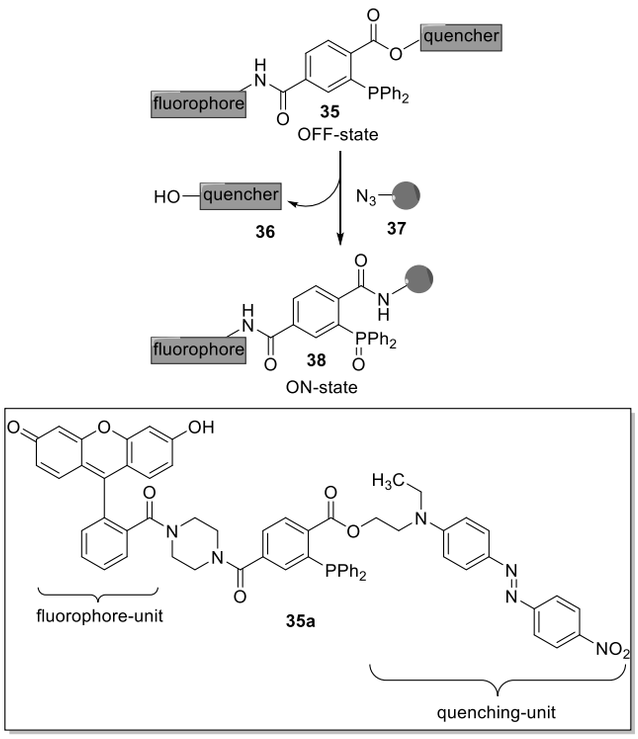
This method may further be applied for the general synthesis of amide bonds for other molecules.[255,258,290]

An advanced and semisynthetic approach deals with the assembly of larger and chemically modified proteins.[275,329,330] This strategy is referred to as the so called expressed protein ligation (EPL). It is characterized by the fact that the peptide fragments are build up by means of biotechnological or chemical synthesis and are then linked immediately by chemical ligation. One of the methods was designed by Raines and co workers.[275]

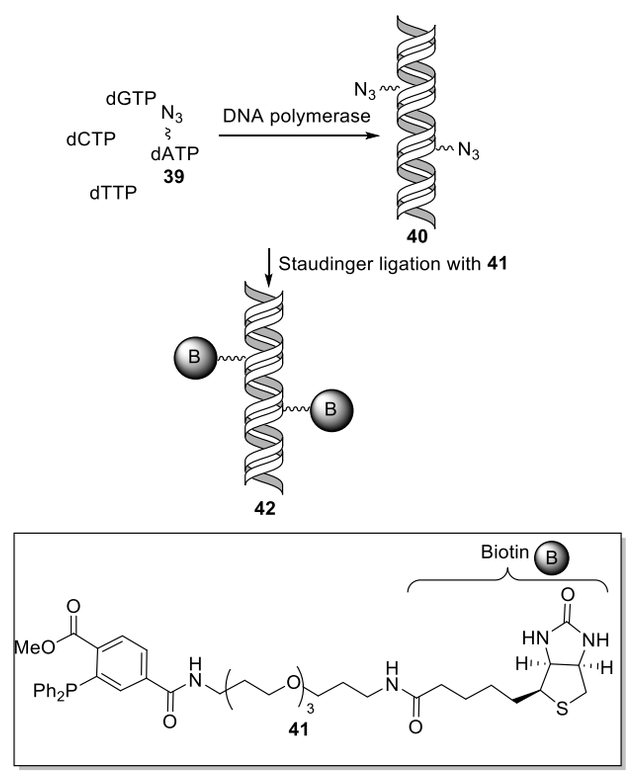
In Scheme 11, an additional application of the Staudinger Ligation is depicted: the peptide cyclization.

Originally, this method was used by Hackenberger et al. for the synthesis of acylated amino sugars and peptides as well as for cyclization of bifunctionalized azido phosphino thioesters **65**.[67,331] By using borane protected phosphinomethane thiol **54**, in which the phosphine is stabilized against oxidation, the reaction with azides (**59**) is triggered after in situ deprotection, and this technique can be used for peptide cyclization as well (Scheme 11A).[133,134,261–263,331,332] The application of the presented method in biological systems is not suitable due to harsh deprotection reaction conditions. Therefore, new strategies have been developed, which are presented in Scheme 11B,C. First, TFA can be used at room temperature to generate medium sized azidopeptide phosphinothioesters **66**. Here, both the borane as well as the protecting group of the peptide side chain are released simultaneously.[261] However, for the acidic deprotection route, it is important to note that the addition of a base such as DIPEA is essential to scavenge residual TFA. Besides, side reactions may occur when lysine side chains are

Scheme 8. Quenched Phosphane Fluorophore Activated upon Staudinger Ligation



Scheme 9. Biotin Labelling of DNA by Staudinger Ligation



used under basic conditions. This may result in the formation of an amide bond with the phosphinothioester.[262]

In addition to cyclization or the coupling of peptides/proteins with each other, it is possible to carry out further modifications by Staudinger Ligation. For example, it succeeded to integrate

Scheme 10. Peptide Ligation of Azide and Phosphinthioester Functionalized Peptides

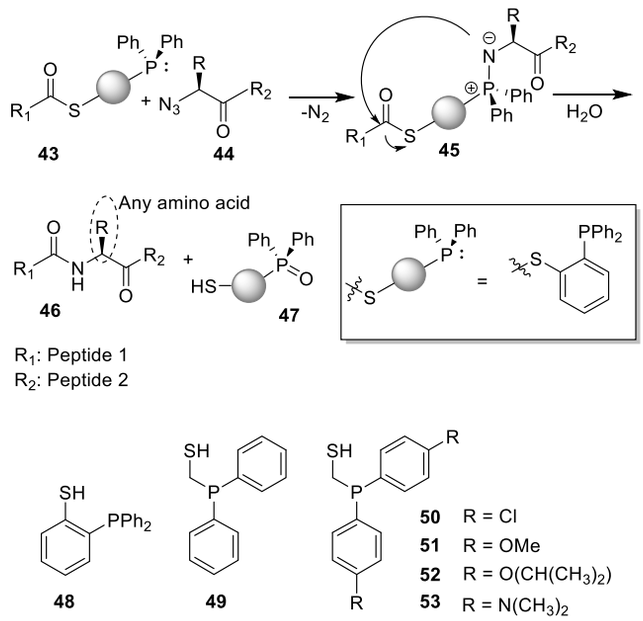


Figure 1. Phosphines for peptide–peptide ligation.

porphyrins with azide functionality so that the final products can eventually be used as models for heme proteins or for ion channels. Furthermore, a strategy for the phosphorylation of proteins was successfully introduced by Hackenberger et al.[311] They used the Staudinger phosphite reaction for the trans formation of azides into phosphoramidates (Scheme 12). The reaction can be carried out both in organic and in aqueous solvents. Starting from an azide **67** and a phosphite, the phosphoramidate **69** is synthesized. Subsequent hydrolysis leads to the formation of phosphoramidate **70**

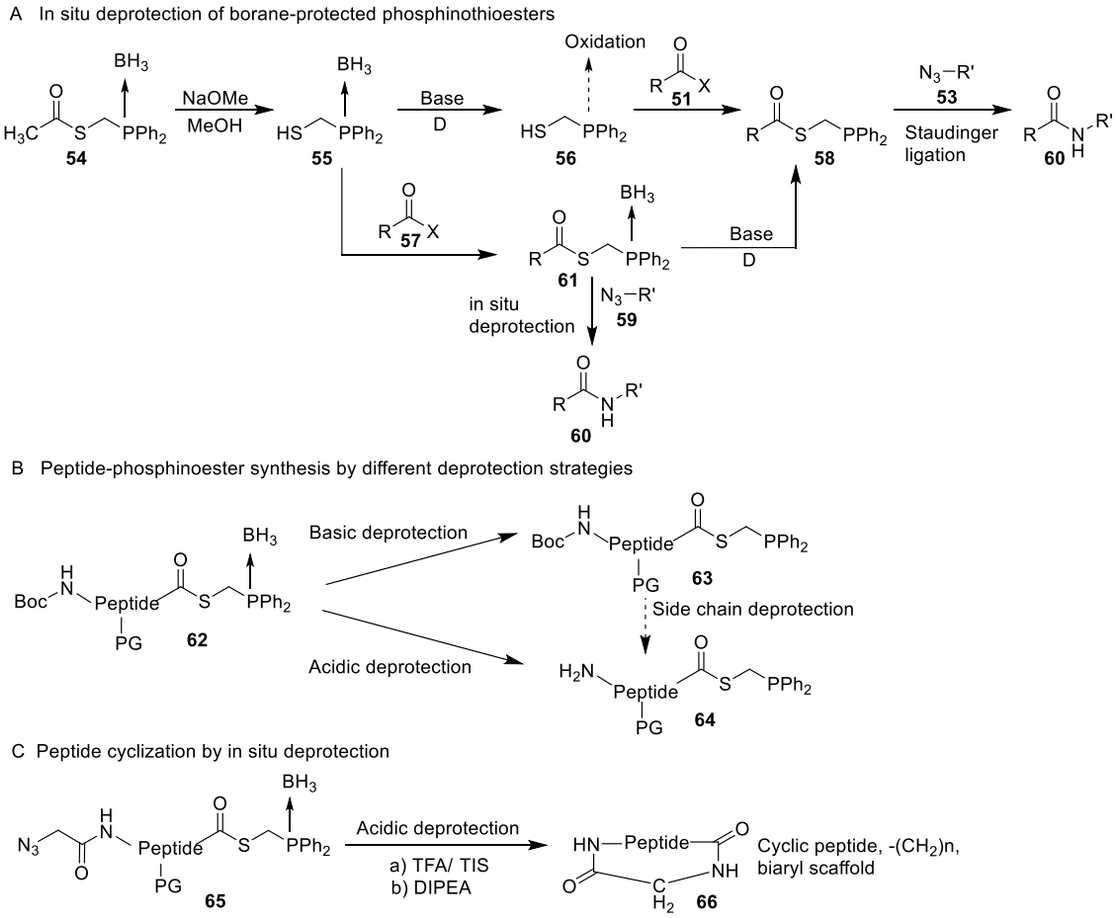
In contrast to the previous descriptions where modifications have been introduced into molecules through Staudinger Ligation, it is also possible to detect post translational modifications of proteins or peptides. Examples include farnesylation,[333] myristoylation,[333] and palmitoylation.[183–189] Additionally, the detection and elucidation of post translational modifications with glycostructures via Staudinger Ligation is a major research area.[9,10,80,192,217,229–231,233,254,334] For some cellular as well as pathological processes, a change in the oxidation state of thiols of cysteine residues in proteins was described.[335] In a novel method, an experimental tool for the labeling of such changes has been introduced. This allows labeling of proteins containing sulfenic acid in living mammalian cells. These may afterward also be isolated and character ized.[198,335]

4.3. Staudinger Ligation for Microarrays and Self-Assembling Systems

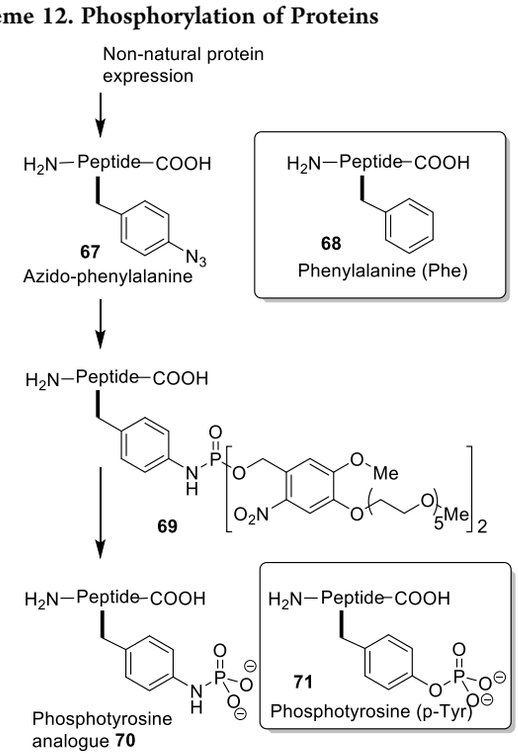
In recent years, the use of microarrays for biological research strongly increased. Hitherto, however, most of these systems are based on DNA. For this reason, it was of great interest to develop such a system on the basis of peptides and small molecules.[52]

Köhn and co workers established the site selective immobi lization of peptides and small molecules on glass surfaces by means of Staudinger Ligation. The generation of these microarrays starts with the modification of the glass surfaces with a phosphine bearing linker. Subsequently, the coupling of

Scheme 11. Peptide Modification Involving the Staudinger Ligation



Scheme 12. Phosphorylation of Proteins



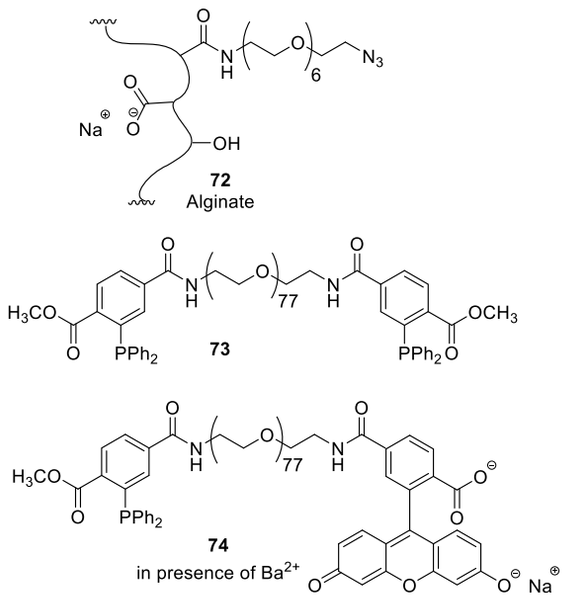
the desired molecules is done via traceless Staudinger Ligation after they have been prepared through solid phase combinatorial synthesis. For this solid phase synthesis, a Kenner type linker is used.[336] Eventually, the covalently bound molecules are exposed on the surface for ligand binding reactions.[337] Overall, this Staudinger based microarray can be prepared under mild reaction conditions and in a water and oxygen containing atmosphere.[52,337] In another approach, the construction of protein microarrays is performed via site selective introduction of azides by means of EPL technology.[338,339]

In addition to these microarray systems this ligation technique is used for the preparation of self assembling monolayers (SAM). One example is the immobilization of RNase A from EPL on a gold surface.[275,298−301,330]

4.4. Metabolic Cell Engineering

Besides the applications described above, the Staudinger Ligation is exploited for metabolic cell engineering, i.e., for the composition of 3D microtissues or for cell surface engineering.[9,220−227,244,245] Inter alia, it is utilized to improve the microencapsulation technique. An ongoing problem with this technology is that the gelation of the conventionally used alginate matrix generally results in a hydrogel which lacks mechanical and chemical stability. The use of the Staudinger Ligation now enables simultaneous increase in stability and reduction of cell toxicity. Moreover, a fluorescent molecule such as carboxyfluorescein can also be integrated into the hydrogel. For the formation of these gels, azide functionalized alginate **72** (Scheme 13) can be covalently coupled to agents like MDT PEG MDT **73** or in the presence of Ba^{2+} to MDT PEG carboxyfluorescein **74** via cross linking. Co incubation of this

Scheme 13. Formation of Fluorescent Hydrogels Using the Staudinger Ligation (Modified from ref 244)



polymer with specified cells, i.e., insulin producing pancreatic cells, results in the final microencapsulation system, with which cells can be transplanted into a host immune system. This technique is currently in studies on applications for treatment of type I diabetes mellitus[340−342] or Parkinson's disease.[343]

As already mentioned, the Staudinger Ligation was also applied for cell surface engineering. This was first described by Bertozzi et al.[9] In this process, glycostructures were marked on cell surfaces while, e.g., a sialic acid precursor, peracetylated *N* azidomannosamine, was prepared, added to, and metabolized by cells. With that, the azide is integrated into the complex glycostructures of the cell's surface. Thereafter, it can be linked with a biotinylated molecule through Staudinger Ligation. This approach could be used, i.e., to inhibit enzymes after chemical modification.[216]

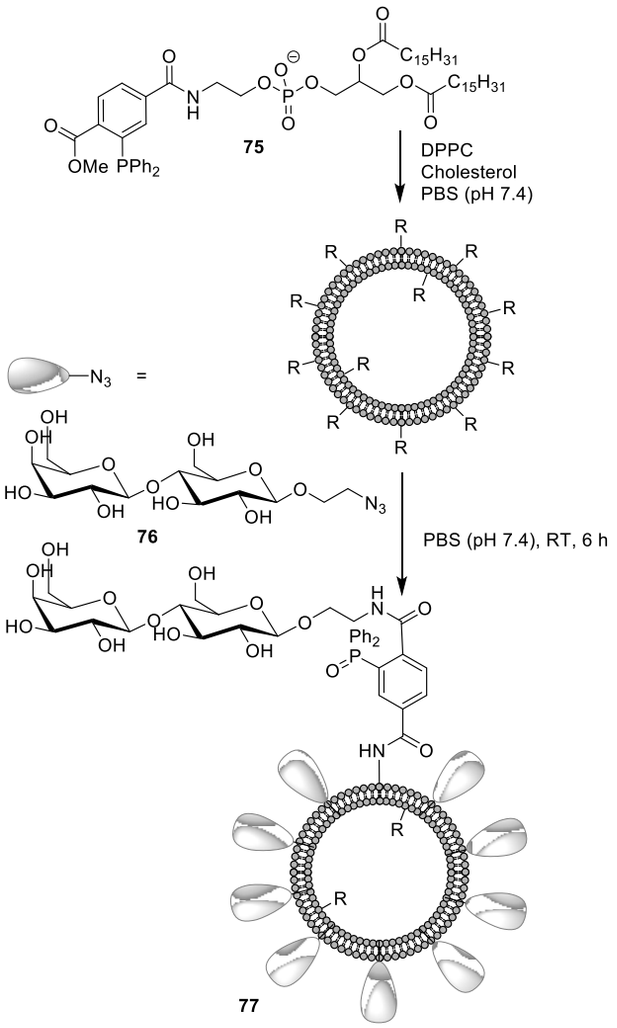
4.5. Staudinger Ligation in Drug Delivery

Furthermore, the Staudinger Ligation is applied for modification of nanoparticles or liposome surfaces. It is advantageous that these linked ligands may be specific for certain surface molecules, especially receptors, in cellular systems. Thus, certain reactions may be triggered or inhibited. Conventional nano particles for this application are composed of polymers like poly L lysine or polyamidoamines. The latter can be present as linear or dendrimeric structures. The introduction of a targeting ligand via Staudinger Ligation was investigated, e.g., by Chan and co workers. They used an *N* acylated PEGylated polyamidoamine in which the azidoacetyl unit was introduced through a carbamic anhydride moiety. This creates an acid labile linker that can be cleaved in the acidic environment of endosomes/lysosomes, resulting in a release of the particles. Finally, coupling of an RGD peptide unit containing a phosphinothioester function ality to the polymer is performed.[297]

The surfaces of liposomes can be modified in a similar manner. One approach for this purpose was developed by Sun and co workers (Scheme 14).[246]

In this case, the functionalization of the surface occurred through an azidolactose moiety **76**, which is coupled to a phosphine functionalized lipid **75**. This special surface pattern

Scheme 14. Surface Modification of Liposomes

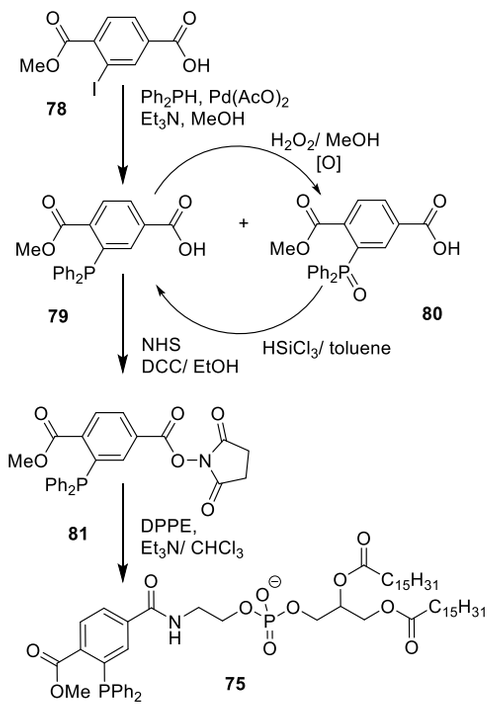


77 represents a model carbohydrate from the glycosphingolipid family, the lactosylceramide. The phosphine functionalized lipid **75** can be generated using the synthesis shown in Scheme 15.

4.6. Staudinger Ligation in Living Animals

In the field of research on live animals, again Bertozzi and co workers pioneered the application of Staudinger Ligation. Investigated biomolecules were predominantly glycostruc tures.[128,129] Intravenous injection of azide functionalized monosaccharides into mice was mainly used for profiling and quantification of *O* linked glycoproteins. These glycosylation patterns of living tissues may differ greatly from individual cells. This is due to a change in the glycome because of a different environment[344] as by developmental processes[80,129] or cell differentiation.[345] Nevertheless, the in vivo technology using Staudinger Ligation needs to be improved even further. For example, there are no reports on visualization of glycostructures using animal imaging so far.[228] However, when phosphanes are used in in vivo studies, their toxicity to living animals has to be considered.[122] For example, triphenylphosphane has an LD_{50} value of 700 mg/kg for oral uptake and an LC_{50} value of 1135 ppm for uptake by inhalation in rats.

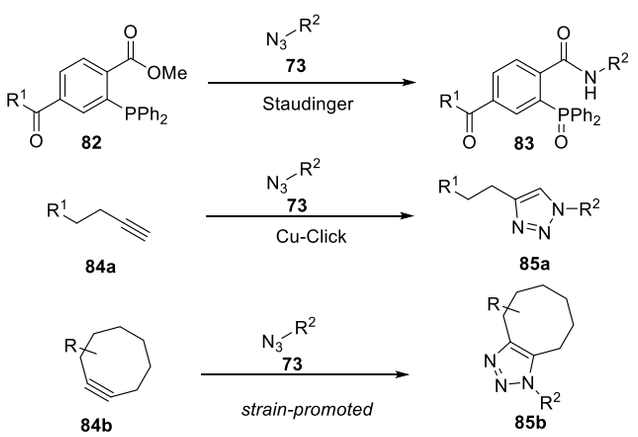
Scheme 15. Synthesis of Phosphine Functionalized Lipids



5. COMPARISON WITH OTHER METHODS

In comparison to azide functionalities with other functional groups in molecules, such as aldehydes or ketones, the former emerge as particularly favorable in the exploitation as bioorthogonal chemical entities. This means they combine the advantages of oxidation resistance, reactivity in water, and especially they do not react with other naturally occurring functional groups in biological systems such as amines or other nucleophiles.[68] Accordingly, azides are particularly well suited for bioconjugation reactions. One of the most common reactions for this is the Staudinger based reaction, which is used in the traceless or phosphite based variant. Here, the use of phosphanes **82** yields amides **83**. Second, the [3 + 2] cycloaddition is a widely used bioconjugation method. In this case, alkynes are converted to triazoles. Both copper catalyzed and copper free reaction conditions are possible. These two reactions are compared in Scheme 16.

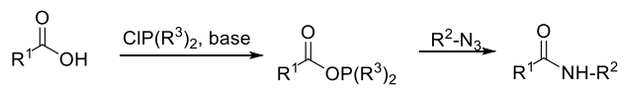
Scheme 16. Comparison of Staudinger Based Reaction and Two [3 + 2] Cycloadditions



Comparing the applicability of these reactions for in vivo issues, the Staudinger Ligation is generally beneficial because it completely avoids toxic copper reagents.[7] At this point, it should be noted that this can circumvented by the use of strained dipolarophiles (e.g., cycloalkynes like **84b**, SPAAC)[346] or by the light induced addition, for example, with tetrazines.[347]

Besides, the generation of amides is also relevant for other scientific issues. Amides can be produced by a multitude of different methods, which are not discussed in detail.[348] Some of them make use of azides and/or phosphanes. A recent report shows that chlorophosphites can mediate the reaction of carboxylic acids and azides to yield amides (Scheme 17) (Table 6).[312]

Scheme 17. Carboxylic Acid−Azide Functionalization



A major application area for these amides is the synthesis of peptides or proteins. In addition to classical biotechnological approaches for their preparation, chemical syntheses, however, have the advantage that they allow introduction of non native amino acids or other types of modifications. Currently, the solid phase synthesis is the most common chemical peptide synthesis method. However, drawbacks are the limited peptide length of 40−50 amino acids and a decreasing yield with increasing chain lengths. At the same time, the number of byproducts increases in the reaction mixture. Therefore, many research efforts toward an optimized chemical peptide synthesis are devoted. For example, different ligation methods have been established such as the classical native Ligation (invented by Wieland[349] and redefined by Kent[350]) and other chemical ligations (expressed chemical ligation[351]).[39] Wieland and co workers[352] introduced an intramolecular rearrangement reaction, which allowed coupling of small peptide fragments in solid phase. With this preliminary work, the "active ester" method was established. This enabled synthesis of protected peptide fragments in liquid phase. Thereupon, the native chemical ligation (NCL) of unprotected peptide fragments was developed.[350] However, this reaction is limited because it is dependent on terminal cysteine residues. Advancements of NCL, which are not based on terminal cysteine, are, e.g., the conformationally assisted ligation[353] and ligations with removable auxiliaries.[8,69,354,355]

When it comes to bioconjugation methods, the thiol−ene and thiol−yne reactions can be considered also as suitable conjugation methods to be compared to the Staudinger Ligation. Interestingly, there are very few reports for their application in vivo,[356] which is due to the fact that thiyl radicals formed during this reaction are trapped by cellular components. However, these reaction are basically orthogonal to azides and phosphines, making them attractive for the construction of complex conjugates.

Over the past decade, a large number of photobioconjugation methods have been emerged with organic azides. Besides classical[357] photoaffinity labeling[358] with azides,[359] also quite complex constructs have been used to attach probes to biological compartments.

6. INTERMOLECULAR REACTIONS

Solely intermolecular reactions are normally not considered as ligation methods, however, in Table 6, we list some examples

Table 6. Other Staudinger Reactions: External Phosphites and Chlorophosphites[77,312]

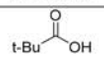
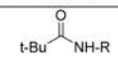
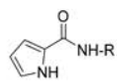
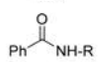
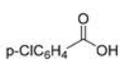
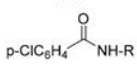
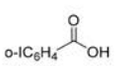
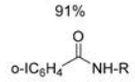
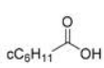
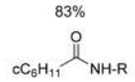
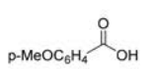
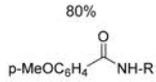
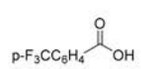
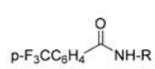
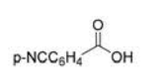
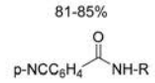
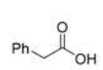
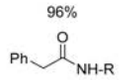
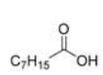
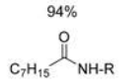
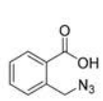
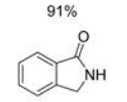
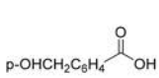
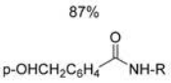
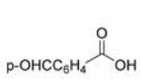
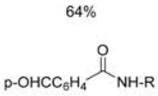
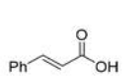
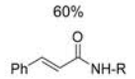
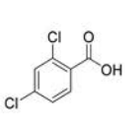
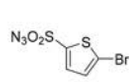
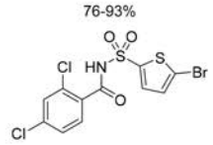
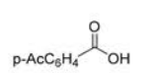
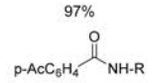
Electrophile	Phosphite	Azide RN_3	Conditions	Product(s) and Yield(s)
	ClP(pin)	R = Ts	NaH, PhCl, 80 to 130 °C, 12 h	 56%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 81%
H-Pro-OH	$ClPPh_2$	$N_3CH_2CO_2Et$	Et_3N, PhCl, 80 to 130 °C, 12 h	*c*-Pro-Gly 62%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 93%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 91%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 83%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 80%
	ClP(pin)	R = Ph, Bn, Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 63-96%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 81-85%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 96%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 94%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 91%
	ClP(pin)	-	Et_3N, PhCl, 80 to 130 °C, 12 h	 87%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 64%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 60%
	ClP(pin)	R = Ts, Ms, p-$MeOC_6H_4$	Et_3N, PhCl, 80 to 130 °C, 12 h	 76-93%
	ClP(pin)		Et_3N, PhCl, 80 to 130 °C, 12 h	 97%
	ClP(pin)	R = Ts	Et_3N, PhCl, 80 to 130 °C, 12 h	 82%

Table 6. continued

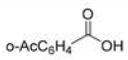
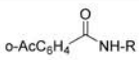
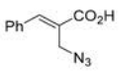
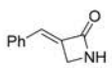
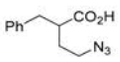
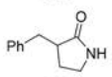
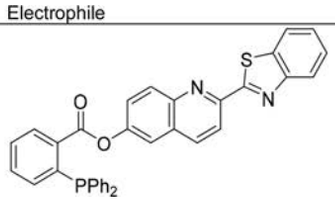
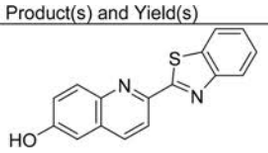
Electrophile	Phosphite	Azide RN_3	Conditions	Product(s) and Yield(s)
	ClP(pin)	R = Bn	Et_3N, PhCl, 80 to 130 °C, 12 h	 34%
	ClP(pin)	-	Et_3N, PhCl, 80 to 130 °C, 12 h	 86%
	ClP(pin)	-	Et_3N, PhCl, 80 to 130 °C, 12 h	 64%
Cbz-Ala-OH	$ClPPh_2$	BnN_3	NaH, PhCl, 80 to 130 °C, 12 h	Cbz-Ala-NHBn 60%
Fmoc-Ala-OH	$ClPPh_2$	$N_3CH_2CO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Ala-Gly-OEt 88%
Fmoc-Pro-OH	$ClPPh_2$	R = CH_2CO_2Et, $CHBnCO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Pro-CONHR 83-90%
Fmoc-Ile-OH	$ClPPh_2$	$N_3CH_2CO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Ile-Gly-OEt 51%
Fmoc-Phe-OH	$ClPPh_2$	R = Ts, CH_2CO_2Et, $CHBnCO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Phe-NHR 70-87%
Fmoc-Phe-OH	$ClPPh_2$	R = Ts, CH_2CO_2Et, $CHBnCO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Phe-NHR 70-87%
Fmoc-Trp(Boc)-OH	$ClPPh_2$	R = Ts, CH_2CO_2Et, $CHBnCO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Trp(Boc)-CONHR 38-78%
Fmoc-Cys(Trt)-OH	$ClPPh_2$	$N_3CH_2CO_2Et$	NaH, PhCl, 80 to 130 °C, 12 h	Fmoc-Cys(Trt)-Gly-OEt 73%

Table 7. Other Staudinger Reactions: Nitrosyls[318]

Electrophile	Conditions	Product(s) and Yield(s)	Refs.
C_{36} 	HNO H_2O		318

being named "Staudinger Ligation" (which was in fact discovered by the late Leopold Horner).[76] In these cases, the carboxylic acid has to be preactivated, e.g., as benzotriazoyl esters or with activation reagents like DCC or EDS to ensure high reactivity. A catalytic variant using silanes as a reducing agent is known.[77] The reaction of iminophosphoranes with acid chlorides is in fact much older.[78] Alternatively, disulfides or diselenides can be used.[79]

This reaction has been used successfully with a number of aliphatic azides, which can contain also a number of functionalities such as esters, hydroxyls, and halides. The phosphane can be both aliphatic and aromatic. Complex natural products such as cruentaren A,[360] sugars,[361−368] or modified nucleic acids have been synthesized using this reaction (Table 8).

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Notes

The authors declare no competing financial interest.

The following abbreviations, excluding those found in "*The Journal of Organic Chemistry* Standard Abbreviations and Acronyms", are used in the text tables.

Biographies

Christin Bednarek studied food chemistry at the University of Karlsruhe (TH) and received her Ph.D. after working in the group of

Table 8. Other Staudinger Reactions: External Phosphanes[77,147,360−368]

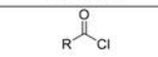
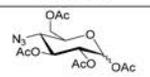
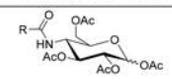
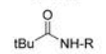
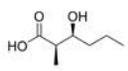
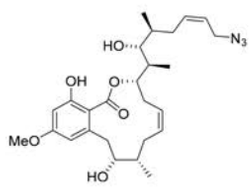
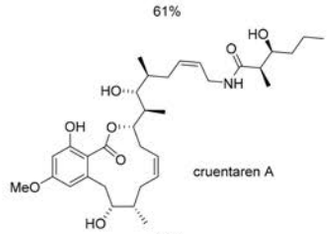
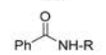
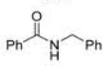
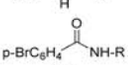
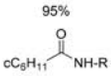
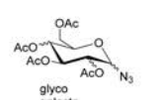
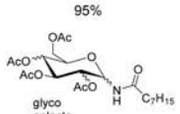
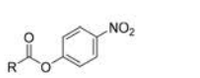
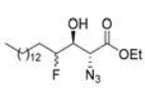
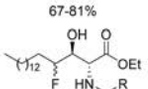
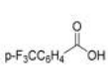
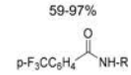
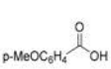
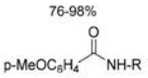
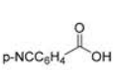
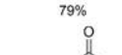
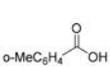
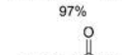
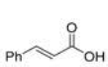
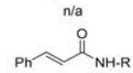
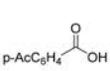
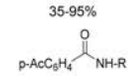
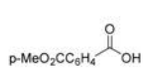
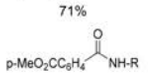
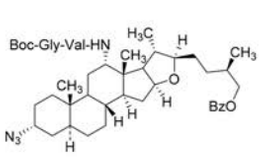
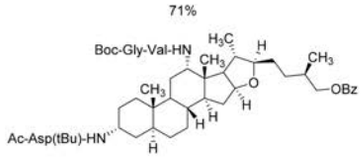
	Electrophile	Phosphane	Azide R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_4 C_6 C_7 C_8	 R = iPr, Pent, Ph, p-$F_3CC_6H_4$	PBu_3 or PPh_3		CH_2Cl_2, reflux	 37-67%	361
C_5		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 110 °C	 61%	77
C_7		PPh_3		COMU, DIPEA, DMF	 cruentaren A 79%	360
C_7		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 110 °C	 94%	77
C_7		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$ PhMe, 111 °C		147
C_7		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 110 °C	 95%	77
C_7		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 110 °C	 95%	77
C_8		PBu_3	 glyco galacto manno	DIC, HOBt, THF, reflux	 glyco galacto manno 67-81%	362
C_8 C_{10} C_{14} C_{18}	 R = CH_3, C_3H_7, C_7H_{15}, $C_{11}H_{23}$	PPh_3		9:1 THF/H_2O, rt, overnight	 59-97%	363
C_8		cat. PPh_3 (10mol%)	R = Bn, $Ph(CH_2)_2$, styrylCH$_2$, furylCH$_2$	$PhSiH_3$, PhMe, 60 °C	 76-98%	77
C_8		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 110 °C	 79%	77
C_8		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 110 °C	 97%	77
C_8		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, dioxane, 50 °C	 n/a	77
C_9		cat. PPh_3 (10mol%)	R = Bn, p-$MeOC_6H_4$, *p*-$MeOC_6H_4CO$	$PhSiH_3$, PhMe, 110 °C	 35-95%	77
C_9		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 60 °C, 18 h	 71%	77
C_9		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, PhMe, 60 °C, 18 h	 71%	77
C_{10}	Ac-Asp(tBu)-OH	PMe_3		THF, then H_2O	 76%	364

Table 8. continued

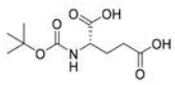
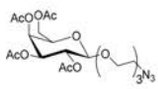
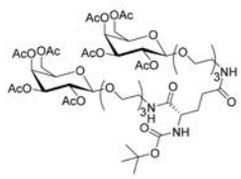
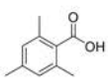
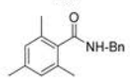
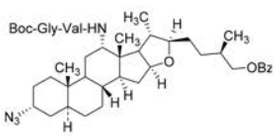
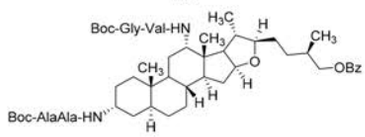
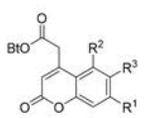
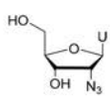
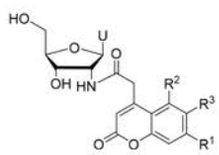
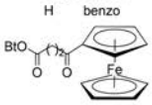
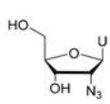
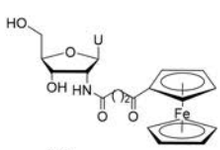
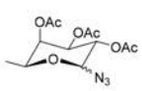
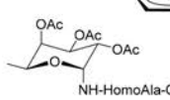
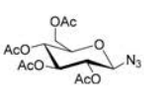
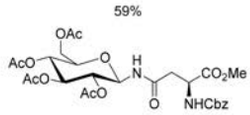
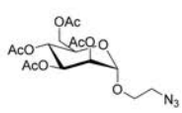
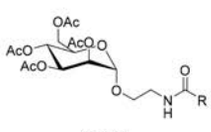
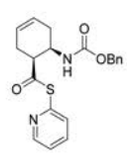
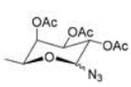
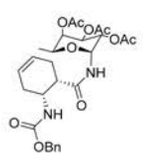
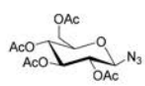
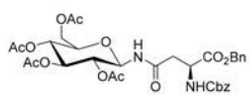
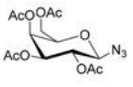
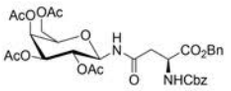
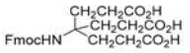
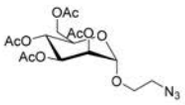
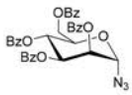
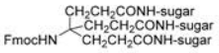
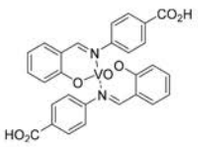
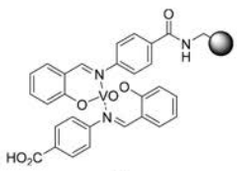
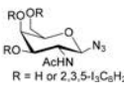
	Electrophile	Phosphane	Azide R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{10}		PBu_3		HBTU, DIPEA, DMF, 4 °C	 65%	361
C_{10}		cat. PPh_3 (10mol%)	R = Bn	$PhSiH_3$, dioxane, 50 °C	 Nd	77
C_{11}	Boc-Ala-Ala-OH	PMe_3		THF, then H_2O	 76%	364
C_{11}	Cbz-Ala-OH	cat. PPh_3 (10mol%)	$N_3CH_2CO_2Et$	$PhSiH_3$, PhMe, 80 °C	Cbz-Ala-Gly-OEt 59%	77
C_{17} C_{18} C_{21} C_{20}	 R^1 R^2 R^3 OH H H OMe OMe H H benzo	PPh_3		9:1 THF/H_2O, rt, overnight	 59-97%	328
		PPh_3		9:1 THF/H_2O, rt, overnight		328
C_{16}	Cbz-HomoAla-S-2pyr	PPh_3		$CuCl_2$, $EtNO_2$, 40 °C	 NH-HomoAla-Cbz	365
C_{17}	Cbz-Phe-OH	cat. PPh_3 (10mol%)	$N_3CH_2CO_2Et$	$PhSiH_3$, PhMe, 80 °C	Cbz-Phe-Gly-OEt 59%	77
C_{18}	Cbz-Asp-(SPy)-OMe	PPh_3		$CuCl_2$, $EtNO_2$, 40 °C		366
C_{19} C_{23} C_{24} C_{37}	Fmoc-Asp-OH Fmoc-Asp(OH)-OtBu Fmoc-Glu-(OH)-OtBu Fmoc-Cys(Trt)-OH	PBu_3		DIC, HOBt, THF, reflux	 32-72%	362
C_{20}		PPh_3		$CuCl_2$, $EtNO_2$, 40 °C		365
C_{24}	Cbz-Asp-(SPy)-OBn	PPh_3		$CuCl_2$, $EtNO_2$, 40 °C	 65%	366

Table 8. continued

	Electrophile	Phosphane	Azide R-N_3	Conditions	Product(s) and Yield(s)	Refs.
C_{24}	Cbz-Asp-(SPy)-OBn	PPh_3		$CuCl_2$, $EtNO_2$, 40 °C		366
C_{25}		PBu_3 or PPh_3	 	DIC, HOBt, THF, reflux	 34-52%	362
C_{28}		PPh_3	 polystyrene or MeO-PEG	benzene, reflux or DMF, 80 °C	 n/a	367, 367
C_n	Carbon nanotube-CO_2H, activated with DCC or EDS	PBu_3	 R = H or 2,3,5-$I_3C_6H_2CO$	DMF, rt, 72 h	Carbon nanotube-CONH-sugar	368

Prof. Dr. Ute Schepers at the Institute of Toxicology and Genetics at the Karlsruhe Institute of Technology. She is a research assistant in the group of Stefan Bräse. Her focus of research is glycochemistry and biology as well as biochemical applications of liposomes, i.e., for tumor therapy. Other projects include drug delivery in general as well as nanostructures synthesis and applications.

Ilona Wehl was born in Berlin, Germany, in 1990. She received her Ph.D. at the Karlsruhe Institute of Technology (KIT) in 2019 for her work on cell penetrating peptoids for organ specific drug delivery under the supervision of Prof. Ute Schepers. She is currently a research assistant at the Karlsruhe Institute of Technology (KIT) under the supervision of Prof. Stefan Bräse.

Nicole Jung studied chemistry at the University of Frankfurt. In 2004, she obtained her Diploma in chemistry and received her Ph.D. in 2008 at the Karlsruhe Institute of Technology (KIT), Campus South (Prof. Stefan Bräse). She is currently group leader of the Combinatorial Chemistry Platform at the Karlsruhe Institute of Technology (KIT), Campus North. She concentrates on the development of new linker strategies for multifunctional cleavage and fluorine/deuterium labelling and designs new methods for the synthesis of heterocycles on bead.

Ute Schepers was born in Rhede, Germany, in 1966. After her studies in chemistry at the University of Bonn, she received her Ph.D. in 1997, working with Prof. Dr. Konrad Sandhoff. She then moved to Harvard Medical School for a postdoctorate (1998−2000) with Prof. Tom Kirchhausen at the Department of Cell Biology. In 2000, she returned to the Kekulé Institut für Organische Chemie and Biochemie in Bonn to start her independent research. Since 2009, she became an independent group leader at the Institute of Toxicology and Genetics of the Karlsruhe Institute of Technology (KIT) in Karlsruhe, where she finished her habilitation. Her research is focused on combinatorial synthesis for the development of organ specific drug delivery systems and 3D tissue reconstruction.

Stefan Bräse received his Ph.D. in 1995 after working with Armin de Meijere in Göttingen. After postdoctoral appointments at Uppsala University (Jan Bäckvall) and The Scripps Research Institute (K. C. Nicolaou), he began his independent research career at the RWTH Aachen and then at the University of Bonn. Since 2003, he is a full Professor at the Karlsruhe Institute of Technology and since 2012 Director of the Institute of Toxicology and Genetics (now Institute for Biological and Chemical Systems) at the KIT. His research interests include methods in drug discovery, combinatorial chemistry towards the synthesis of biologically active compounds, total synthesis of natural products, and nanotechnology.

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DEDICATION

Dedicated to Professor R. R. Schmidt, a pioneer in glycosylation chemistry, on the occasion of his 85th birthday in 2020.

ABBREVIATIONS USED

ACGT = nucleic acids
AIBN = 2,2′ azobis(isobutyronitrile)
AT = antithrombin
ATP = adenosine triphosphate
BACE1 = beta site APP cleaving enzyme 1
BAIB = [bis(acetoxy)iodo]benzene
Boc = *tert* butoxycarbonyl
BSP = 1 benzenesulfinyl piperidine
BTI = bis(trifluoroacetoxy)iodobenzene
Bz = benzoyl
BzCN = benzoyl cyanide
C_5 epi = C5 epimerase
CAN = ceric ammonium nitrate
Cbz = benzyloxycarbonyl
COMU = (1 cyano 2 ethoxy 2 oxoethylidenaminooxy) dimethylamino morpholino carbenium hexafluorophosphate

CS = chondroitin sulfate
CSA = D,L 10 camphorsulfonic acid
CuAAC = copper catalyzed azide−alkyne cycloaddition
DABCO = 1,4 diazabicyclo[2.2.2]octane
DBU = 1,8 diazabicyclo[5.4.0]undec 7 ene
DCC = *N*,*N*′ dicyclohexylcarbodiimide
DDQ = 2,3 dichloro 5,6 dicyano 1,4 benzoquinone
DIC = *N*,*N*′ diisopropylcarbodiimide
DIPEA = *N*,*N* diisopropylethylamine
DMAC = *N*,*N* dimethylacetamide
DMAP = 4 dimethylaminopyridine
DMEDA = dimethylethylenediamine
DMSO = dimethyl sulfoxide
DMTST = dimethylsulfonium triflate
DS = dermatan sulfate
EcGalU = *Escherichia coli* glucose 1 phosphate uridylyl transferase
EDC = 1 ethyl 3 (3 (dimethylamino)propyl)carbodiimide
^{F}Boc = $F_7C_3C_2H_4C(CH_3)_2OCO$
fCS = fucosylated chondroitin sulfate
FGF = basic fibroblast growth factor
Fmoc = fluorenylmethyloxycarbonyl
FSPE = fluorous solid phase extraction
Fuc = fucose
GAG = glycosaminoglycan
GalA = galactosic acid
GalNAc = *N* acetylgalactosamine
GDP = guanidine diphosphate
GlcA = glucuronic acid
GlcA = glucuronic acid
GlcN = glucosamine
GlcNAc = *N* acetylglucosamine
GlcNS = *N* sulfoglucosamine
GlcNTfa = *N* trifluoroacetylglucosylamine
GPI = glycosylphosphatidyl inositol
HA = hyaluronan
HBSF buffer = 20 mM HEPES, 150 mM NaCl, and 1% FBS, pH 7.4
HOBt = 1 hydroxybenzotriazole
HP = heparin
HPLC = high performance liquid chromatography
HPPG = heparin proteoglycans
HS = heparan sulfate
IdoA = iduronic acid
Im = imidazole
IMMS = ion mobility mass spectrometry
K_d = dissociation constant
KfiA = *Escherichia coli* glycosyltransferase
KS = keratan sulfate
LacNAc = *N* acetyllactosamine
Lev = levanoyl
LevOH = levulinic acid
LHMDS = lithium bis(trimethylsilyl)amide
LMWH = low molecular weight heparins
MBz = *para* methylbenzoyl
Mca = monochloroacetyl
*m*CPBA = *meta* chloroperoxybenzoic acid
MeCN = acetonitrile
MES = 2 (*N* morpholino)ethanesulfonic acid
MOMCl = methoxymethyl
MP = *para* methoxyphenyl
Ms = mesyl
MS = molecular sieves
MUF = 4 methylumbelliferyl
NAD = nicotinamide adenine dinucleotide
NAP = 2 naphthylmethyl
NBS = *N* bromosuccinimide
Neu5Ac = *N* acetylneuraminic acid
NFU = National Formulary Units
NHS = *N* hydroxysuccinimide
NIS = *N* iodosuccinimide
OST = *O* sulfotransferase
PAMAM = poly(amidoamine)
PAPS = 3′ phosphoadenosin 5′ phosphosulfate
PBB = *p* bromobenzyl
PBH = pyrenebutyric acid hydrazide
PDC = pyridinium dichromate
PEG = polyethylene glycol
Phth = phthalimide
PivOH = pivalic acid
PMB = *para* methoxybenzyl
PmGlmU = *Pasteurella multocida N* acetylglucosamine 1 phosphate uridylyltransferase
PmHS2 = *Pasteurella multocida* heparosan synthase 2
PmPpA = *Pasteurella multocida* inorganic pyrophosphatase
PmUgd = *Pasteurella multocida* UDP glucose dehydrogenase
*p*TolSCl = 4 methyl benzenesulfenyl chloride
p TsCl = *para* toluenesulfonyl chloride
p TsOH = *para* toluenesulfonic acid
ROMP = ring opening metathesis polymerization
RP HPLC = reversed phase high performance liquid chromatography
SE = 2 (trimethylsilyl)ethyl
Ser = serine
SPAAC = strain promoted azide−alkyne cycloaddition
Su = succinyl
TBABr = tetra *N* butylammonium bromide
TBAF = tetra *N* butylammonium fluoride
TBAI = tetra *N* butylammonium iodide
TBAN = tetra *N* butylammonium nitrate
TBDMS = *tert* butyldimethylsilyl (preferred over TBS)
TBDMSOTf = *tert* butyldimethylsilyl trifluoromethanesulfonate
TBDPS = *tert* butyldiphenylsilyl
TCA = trichloroacetic acid
Tca = trichloroacetyl
TCE = 2,2,2 trichloroethyl
Tci = trichloroacetimidyl
TDS = (dimethyl)thexylsilyl
TEG = tetraethylene glycol
TEMPO = 2,2,6,6 tetramethylpiperidinyloxy
Tes = triethylsilane
TES = triethylsilyl
TFA = trifluoroacetic acid
Tfa = trifluoroacetyl
TFAA = trifluoroacetic acid anhydride
TfOH = trifluoromethanesulfonic acid
TMA = trimethylamine
TMS = trimethylsilyl
$TMSN_3$ = trimethylsilyl azide
TMSOTf = trimethylsilyl trifluoromethanesulfonate
Tol = tolyl
Tr = trityl
Tris = tris(hydroxymethyl)aminomethane
Troc = 2,2,2 trichloroethoxycarbonyl
TSAS = transition state analogue substrate

TTBP = 2,4,6 tri*tert* butylpyrimidine
UDP = uridine diphosphate
UMP = uridine monophosphate
UTP = uridine triphosphate

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