New Protecting Group Strategies for the Solid-Phase Synthesis and Modification of Peptides, Oligonucleotides, and Oligosaccharides

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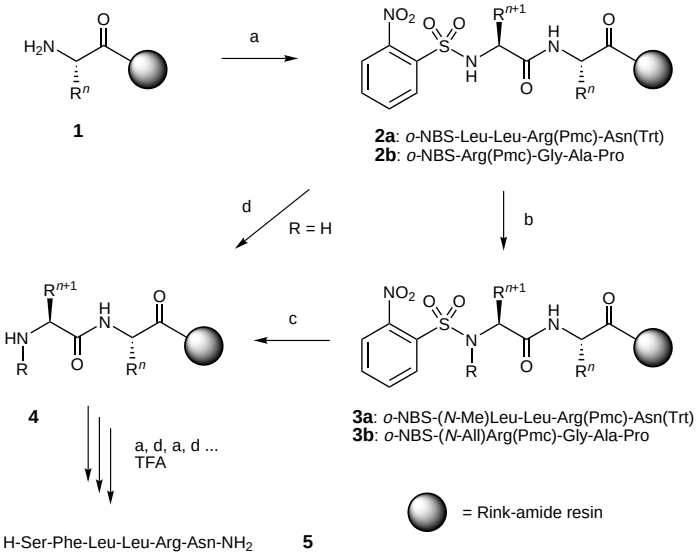
Merrifield's pioneering invention of solid-phase peptide synthesis[1] revolutionized the field of biological and medicinal chemistry. The solid-phase synthesis of peptides and oligonucleotides quickly reached a high level of maturity that allowed the repetative process to be automated. This enabled non-chemists to readily synthesize these complex biopolymers and allowed the first detailed investigations of the structure–affinity relationships of peptides and oligonucleotides. The efficiency of the automated process led many to the assumption that all problems had been solved and that new developments would be superfluous. However, the increasing pressure in pharmacological research to find more lead structures in even shorter periods of time has rejuvenated solid-phase methods, as demonstrated by the developments in combinatorial chemistry. Not only has this facilitated the preparation of peptides and oligonucleotides, it has also encouraged research into the solid-phase synthesis of highly complex oligosaccharides. Here new strategies for the solid-phase synthesis of these three classes of biopolymers are presented. The focus is on the use of activating protecting groups which increase both orthogonality and diversity.

Miller et al. utilized the acidifying effect of the *ortho*-nitrobenzenesulfonyl group (*o*-NBS) for the activation and selective N-alkylation of amido groups.[2] The incorporation of N-alkylated amino acids has often been used to probe the bioactive conformation of a peptide. To perform N-alkylations on a solid-phase, amino acids with N-*o*-NBS protecting groups were coupled to the unprotected amino group of a peptide **1** (Scheme 1).[3] Subsequently, the N-*o*-NBS peptide derivatives **2** were selectively alkylated at the acidic sulfonamide group. For example, reaction of the supported tetrapeptide **2a** with methyl 4-nitrobenzenesulfonate and the base 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD) gave **3a** in quantitative yield. The Pd^0-catalyzed N-allylation of **2b** succeeded with allylmethyl carbonate in a yield of 98%. The *o*-NBS group was cleaved by treating the tertiary sulfonamides **3** with sulfanylethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Under these conditions, the unalkylated secondary sulfonamides remained intact.

The *o*-NBS group was also used as a general temporary protecting group in the solid-phase synthesis of unalkylated peptides. In the cleavage step, **2** was treated with 0.5 M potassium thiophenolate for 10 min; this liberated the amino group of **4** and a yellow chromophore. Thus, the course of the cleavage reaction can be conveniently monitored by photometry. The *o*-NBS and Fmoc (Fmoc = 9-fluorenylmethoxycarbonyl) strategies[4] were compared in the synthesis of the thrombine receptor agonist **5**. Purification of the crude products, which were obtained in 85% (*o*-NBS) and 91% (Fmoc) purity, by HPLC gave the pure peptide **5** in 50% (*o*-NBS) and 62% (Fmoc) overall yield.



Scheme 1. a) 4 equiv *o*-NBS-NH(CHR^{n+1})COOH, 3.8 equiv HBTU, NMM, DMF, 20 min; b) R = Me: 4 equiv p-O_2NPhSO_3Me, 3 equiv MTBD, DMA, 30 min; R = All: 15 equiv AllOCOOMe, 10 mol% [Pd_2dba_3], $CHCl_3$, 80 mol% PPh_3, THF, 2 h; c) 10 equiv $HO(CH_2)_2SH$, 5 equiv DBU, DMF, 30 min; d) 0.5 M PhSH, 2 equiv K_2CO_3 per PhSH, DMF, 10 min. All = allyl, dba = dibenzylideneacetone, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMA = *N*,*N*-dimethylacetamide, HBTU = 2-((1*H*)-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NMM = *N*-methylmorpholine, MTBD = 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene, *o*-NBS = *ortho*-nitrobenzenesulfonyl, Pmc = 2,2,5,7,8-pentamethylchromane-6-sulfonyl, TFA = trifluoroacetic acid, Trt = trityl.

Protecting groups that allow the introduction of additional functional groups after a solid-phase synthesis are of particular interest for the construction of medically relevant DNA and RNA conjugates. In contrast to proteins, only a few functional groups of nucleic acids can be used for the conjugation of reporter groups or cross-links without compromising their biological function. In 1990, Verdine and MacMillan reported a method in which uridine derivatives were incorporated into oligonucleotides and subsequently modified.[5] This concept was recently extended to the synthesis of functionalized oligoribonucleotides.[6] The inosine base **6** and the uridine base **8** bear *para*-chlorophenyl groups at O6 and O4. Simple substitution with primary amines (RNH_2) resulted in the formation of the corresponding adenosine and cytidine derivatives (Scheme 2). Thus, the R group can be introduced into the major groove of a nucleic acid duplex. After substitution of the fluoride and cleavage of the nitrophenylethyl (NPE) protecting group, the fluoroinosine **7** forms a guanosine system, in which the the R group is located in the minor groove.

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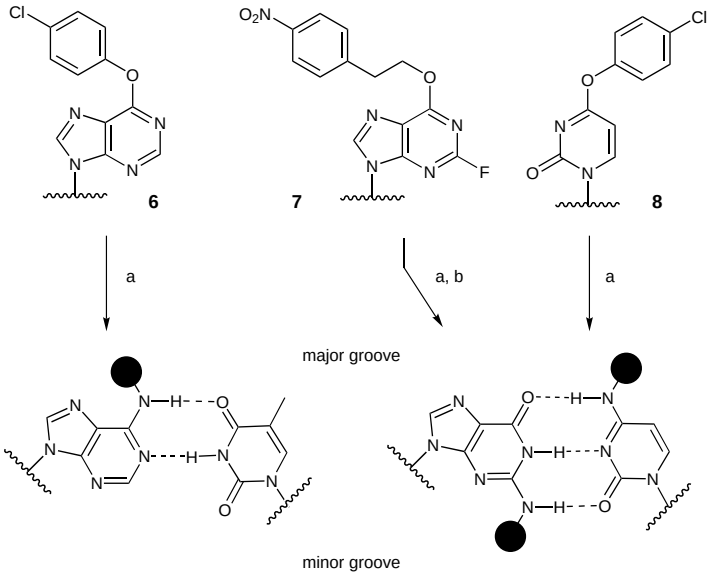
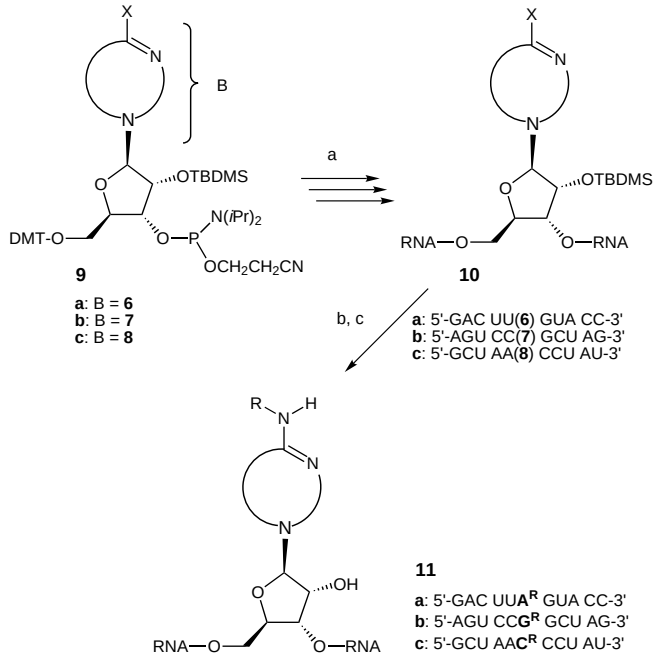


Scheme 2. Location of the R group in the duplex after reaction of the modifiable nucleosides **6**, **7**, and **8** with primary alkylamines a) RNH_2, MeOH; b) $NEt_3 \cdot 3HF$.

For the synthesis of the RNA-oligomers **10a–c** the phosphoamidites **9a–c** were incorporated by means of a slightly modified RNA synthesis protocol (Scheme 3). The



Scheme 3. Convergent modification of RNA. a) Solid-phase RNA synthesis by the phosphoamidite strategy; b) 2 M RNH_2 in MeOH (R = Me, 8 M CH_3NH_2 in MeOH); c) **10a**→**11a**, **10c**→**11c**: 1 M TBAF in THF; **10b**→**11b**: $NEt_3 \cdot 3HF$. DMT = 4,4′-dimethoxytrityl, TBDMS = *tert*-butyldimethylsilyl.

oligomers **10a–c** were functionalized with the amines listed in Table 1. Subsequent treatment with tetra-*n*-butylammonium fluoride (TBAF) or $NEt_3 \cdot HF$ removed the nitrophenylethyl (NPE) and *tert*-butyldimethylsilyl (TBDMS) protecting groups. The relative yields were evaluated after purifying

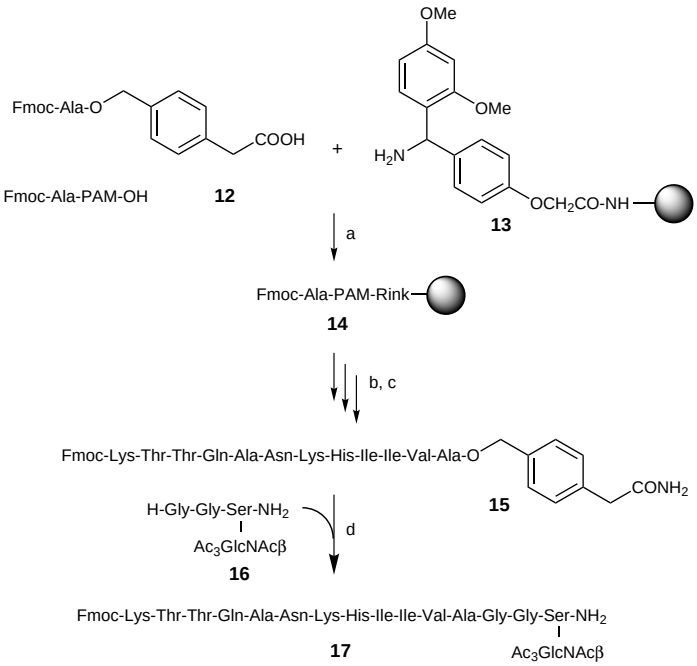
Table 1. Relative yields of substitutions of **10a–c** with RNH_2 to give **11a–c** (Scheme 3).

R	**a**[a]	**b**[b]	**c**[b]
H	0.24	1.0	1.0
CH_3	1.0	0.85	1.0
$H_2NCH_2CH_2$	1.0	0.9	0.96
$H_2N(CH_2)_4$	1.04	0.88	1.0
$HOCH_2CH_2$	1.1	1.0	1.04
$PhCH_2$	0.67	0.65	0.79

[a] Relative to the reaction with methylamine. [b] Relative to the reaction with ammonia.

the products **11a–c** by denaturing polyacrylamide gel electrophoresis (PAGE) and enzymatic hydrolysis. Finally, the composition of the nucleic acid was determined by HPLC. All conversions proceeded in almost quantitative yield, with the exception of the reactions with benzylamine and of **10a** with ammonia.

The postsynthetic activation of a peptide ester was reported by Wong et al.[7] For the synthesis of a partial sequence of the C-terminal region of ribonuclease B, conjugate **12**, consisting of N-Fmoc-protected alanine and the acid- and base-stable phenylacetamidomethyl (PAM) linker, was coupled to the Rink amide resin **13** (Scheme 4). The elongation of **14** followed standard Fmoc protocols.[4] Standard TFA cleavage conditions removed all acid-labile protecting groups from the side chains and also detached the N-Fmoc-protected peptide–PAM ester **15** from the solid support. Since a benzhydrylamine-type linker was used, the peptide–PAM conjugate



Scheme 4. a) 1. HBTU, HOBt, NMM, DMF; 2. Ac_2O, py; b) 1. 20% piperidine/DMF; 2. Fmoc-AA-OH, HBTU, HOBt, NMM, DMF; 3. Ac_2O, py; c) TFA/Et_3SiH/H_2O (95/2.5/2.5), 89%; d) DMF/50 mM triethanolamine (9/1), subtilisin (8397 K 256 Y), 84%. $Ac_3GlcNAc$ = 2-acetamido-3,4,6-tri-*O*-acetyl-2-desoxyglucose, AA = amino acid, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = 2-((1*H*)-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = 1-hydroxybenzotriazole, NMM = *N*-methylmorpholine, TFA = trifluoroacetic acid.

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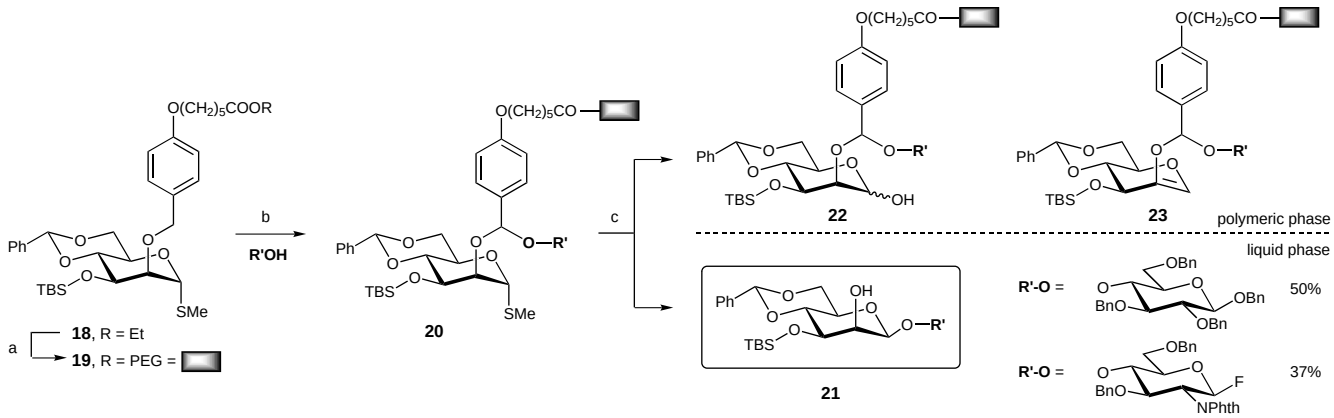

Scheme 5. a) 1. NaOH, *t*BuOH; 2. PEG monomethyl ether, $EtO_2CN{=}NCO_2Et$, PPh_3, CH_2Cl_2, THF, 80%; b) DDQ, 4 Å molecular sieves, CH_2Cl_2, 3 h; c) MeOTf, MeSSMe, 2,6-di-*tert*-butyl-4-methylpyridine, $ClCH_2CH_2Cl$, 4 Å molecular sieves, 21–120 h. Bn = benzyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, PEG = poly(ethylene glycol), Phth = phthaloyl, TBS = *tert*-butyldiphenylsilyl, Tf = triflate.

15 was liberated as a PAM amide. Such peptide esters with unprotected side chains can serve as acyl donors in enzyme-catalyzed peptide couplings. Accordingly, the segment condensation of **15** with the N-terminally unprotected glycotripeptide **16** was catalyzed by the protease subtilisin to give the glycopentadecapeptide **17** in 84% yield. This synthetic strategy is a viable route for the preparation of acid- and base-stable peptide esters. Since these esters are converted to active esters by subtilisin, they can be used in enzymatic segment condensations, as an alternative to chemical processes.[8]

Recently, Ito and Ogawa demonstrated that polymer-bound protecting groups can stereospecifically direct the introduction of a reagent, as shown in the polymer-supported synthesis of β-mannoglycosides.[9] Because of the stereoelectronically unfavorable 1,2-*cis* configuration, the thermodynamically disfavored β-mannosidic bond in **21** (Scheme 5) presents a synthetic challenge. According to a strategy published in 1991 by Barresi and Hindsgaul, the axial 2-OH group can be utilized to direct the aglycon in the glycosylation reaction to the β face.[10] Ito and Ogawa introduced a *para*-alkoxybenzyl protecting group at this position. For the attachment to a polymeric support, the alkoxybenzyl group of the methyl sulfanylmannoside **18** carried a carboxyl group. Oxidation of the polymer-bound mannoside **19** with dichlorodicyanobenzoquinone (DDQ) in the presence of an alcohol yielded the acetal **20**. After activation of the thioglycoside with methyl triflate, **20** underwent intramolecular transacetalization to give the β-mannoside **21**. It is noteworthy that only the desired glycosylation products were liberated from the polymeric resin. Side products such as the hydrolysis product **22** or the elimination product **23** remained on the solid phase.

This example illustrates that protecting groups can be actively used in synthesis and achieve more than temporary blocking of a functional group. The activating effect of protecting groups such as *o*NBS, the PAM amide, and the chlorophenyl group allow subsequent selective reactions to be carried out, the products of which would normally be laborious to synthesize by other routes. The example of the polymer-supported synthesis of β-mannosides demonstrates how activatable protecting groups can initiate stereoselective reactions given a suitable orientation. The latter development is certainly attractive for future research. The polymeric resin could serve as a molecular gatekeeper, which in the final synthetic step liberates only the desired products. Thus, both the syntheses and the purification procedures, which are often time-consuming can be significantly simplified.

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