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Monomethoxytrityl (MMT) as a Versatile Amino Protecting Group for Complex Prodrugs of Anticancer Compounds Sensitive to Strong Acids, Bases and Nucleophiles

Gene M. Dubowchik* and Shilpa Radia

Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 5100, Wallingford, CT 06492-7660

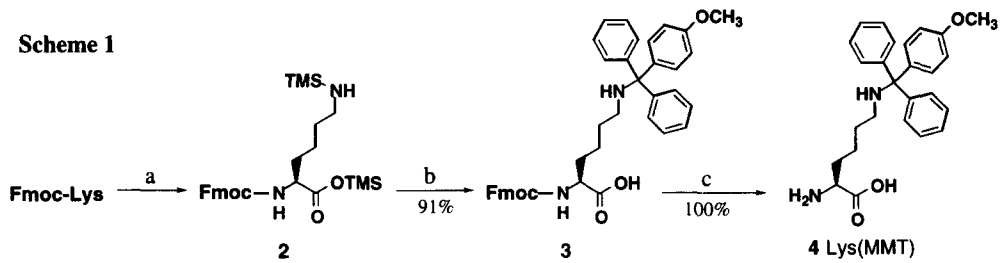
Abstract: Cathepsin B-sensitive maleimidocaproyl-Phe-Lys linker compounds containing acid-sensitive anticancer drugs doxorubicin, mitomycin C and paclitaxel (taxol®) attached through a self-immolative p-aminobenzylcarbonyl spacer were prepared using monomethoxytrityl (MMT) as the amino protecting group for Lys. MMT could be removed cleanly and in high yield by dichloroacetic and chloroacetic acids in the presence of anisole with minimal workup.

Our interest in targeting anti-cancer drugs using monoclonal antibodies[1] that are internalized by tumor cells has led to the development of dipeptide linkers that are stable in circulation but quickly cleaved by lysosomal proteases, releasing free drug.[2] Drug-linker compounds such as **11** (scheme 2) contain a lysine residue that must remain protected during synthesis and then be deblocked in the final step. It is also important that the amino group be protonated very soon after deprotection, otherwise it quickly adds to maleimide, leading to the formation of polymeric by-products. Many of the features of compounds such as **11** make the choice of an amino protecting group very difficult, as they are unstable to such common deblocking conditions as TFA/CH_2Cl_2 (Boc), hydrogenation (Z), $Pd(PPh_3)_4$ (alloc), and secondary amines (Fmoc). Modest success was achieved with the Fmoc group using 1% DBU/THF for < 1 min, followed by quenching with 1M HCl/ether. However, yields of only 30-50% and chromatography on celite or Sephadex® LH-20 were drawbacks.

Because of the sensitivity of **11** to these diverse deblocking conditions we investigated monomethoxytrityl (MMT) as a potentially useful protecting group that could be removed in very mild acidic media. MMT is frequently used in nucleoside chemistry[3] but appears rarely as an amino protecting group.[4] Although MMT-amines are not amide-like and will react with acid chlorides and chloroformates above ca. -40°C in the presence of base, they only react with activated esters, carbonates and alkylating agents under vigorous conditions.

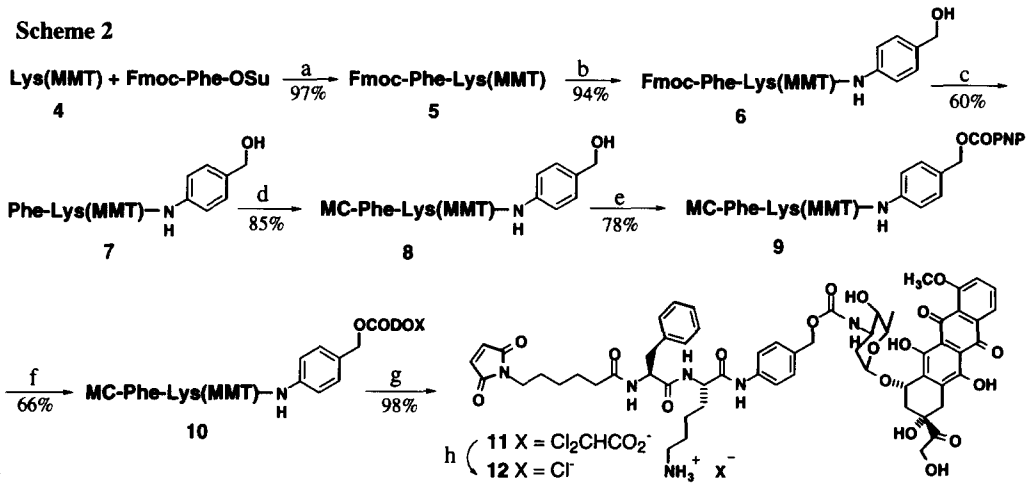
Lys(MMT) **4** was prepared from Fmoc-Lys by a modification of a one-pot tritylation procedure for unprotected amino acids in which both the amino and carboxyl groups are transiently silylated with TMSCl,[5] followed by Fmoc removal using diethylamine (scheme 1).

Scheme 1



(a) i) TMS-Cl, CH_2Cl_2, reflux, 1h, ii) DIEA, 0°C to rt, 1h; (b) i) p-anisyldiphenylmethyl chloride, rt, 18h, ii) MeOH, 40°C, 20 min; (c) Et_2NH, DMF, rt, 1h.

Scheme 2



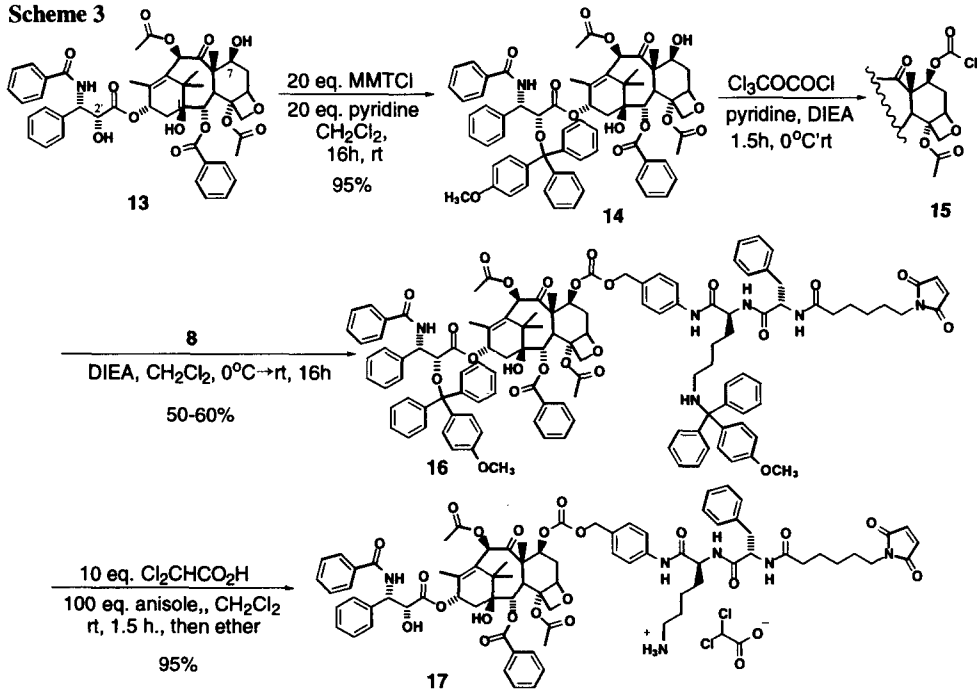
(a) LiOH, DME/water, rt, 18h; (b) p-aminobenzyl alcohol, EEDQ (2 eq.), THF, rt, 18h; (c) Et_2NH, DMF, rt, 1h; (d) MC-OSu, DME, rt, 4h; (e) PNP_2CO, DIEA, sieves, CH_2Cl_2, rt, 18h; (f) DOX•HCl, DIEA, DMF, rt, 2 d; (g) Cl_2CHCO_2H, anisole, CH_2Cl_2, rt, 1h; (h) AG® 2-X8 resin (Cl^-), CH_3OH.

Elaboration of **4** into MC-Phe-Lys(MMT)-PABC-DOX **10** is shown in scheme 2.[6] Only compounds **7** and **10** required purification by silica gel chromatography. The other intermediates could be adequately purified by trituration with ether or mixtures of CH_2Cl_2/ether. The use of the self-immolative p-aminobenzyl carbonyl (PABC) spacer[7] between the dipeptide and drug ensures access to the site of cleavage by lysosomal enzymes.[8] Treatment of **10** with ten equivalents of dichloroacetic acid (0.15-0.2M) in a mixture of anisole (100 equiv.)/CH_2Cl_2 at room temperature effected complete cleavage of MMT within one hour.[9] Chloroacetic acid, in the same proportions, required 3.5-4 hrs, and acetic acid caused incomplete conversion even after 24 hrs.[10] Dilution of the reaction mixture with ethyl acetate, followed by filtration and washing gave **11**, as its dichloroacetate salt, in nearly quantitative yield as an analytically pure orange powder. Since the ultimate fate of these compounds is to be conjugated to macromolecules in aqueous media, enhanced water solubility is advantageous. Exchange of the lipophilic dichloroacetate anion in **11** with chloride was easily effected by stirring with AG® 2-X8 ion exchange resin (Cl^- form, Bio-Rad) in CH_3OH for an hour, followed by filtration, concentration *in vacuo,* and trituration with CH_2Cl_2, or by simply eluting the substrate slowly through a resin-packed column.

Paclitaxel (taxol®) **13**, mitomycin C (MMC) and an extremely potent MMC analogue, BMS-42639, were also linked to our MMT-protected maleimidopeptide.[11] Paclitaxel contains two hydroxyl groups (2' and 7, scheme 3) that can be acylated under comparatively mild conditions (scheme 3). However, esters and carbonates at the 7-position are sterically protected against enzymatic and general hydrolysis in comparison with the 2'-position.[12] The more accessible 2'-hydroxyl was protected as its monomethoxytrityl (MMT) ether **14** by reaction with an excess of MMTCl/pyridine. When a smaller excess was used, or when the reagents were added in portions over several hours, the yield was lower and the reaction stalled. Since the side chain is tucked underneath the taxane core,[13] exposure to a large excess of the bulky protecting reagent all at once is apparently required. Chloroformate formation at the 7-hydroxyl (**15**) was carried out using diphosgene in the presence of pyridine/DIEA. Addition of the maleimido-peptide-benzyl alcohol **8** gave the bis-MMT protected paclitaxel-

dipeptide **16** in good yield (unreacted **14** was easily separated and could be reused). When only pyridine was used as base almost half of the 2'-MMT ether was removed by the pyridinium hydrochloride. No sign of MMT-amine cleavage was observed. An added equivalent of DIEA ensured MMT ether stability. Both protecting groups from **16** were easily removed under the same conditions used for deblocking **10**. Ether was used to precipitate **17** since it is completely soluble in ethyl acetate.[14]

Scheme 3



MMC and its derivatives are much more acid-sensitive than DOX or paclitaxel. When dichloroacetic acid was used to deprotect MMC-containing dipeptide linkers only mitosene degradation products, resulting from elimination of CH_3OH from the MMC core, were isolated. Chloroacetic acid (10 equiv., 0.10-0.15M)/anisole (100 equiv.) in CH_2Cl_2 over 4 h, however, effected the deprotection cleanly and in high yield. Even with an agent as mild as chloroacetic acid care must be taken since large amounts of mitosene was generated when 20 equiv. were used in an attempt to speed up the deprotection.

Besides allowing deprotection under very mild conditions the MMT group also serves as a solubilizing moiety during synthesis. The replacement of MMT with standard carbamate protecting groups such as Boc and Fmoc in scheme 2 generated intermediates that were much less soluble in convenient organic solvents such as CH_2Cl_2, dimethoxyethane and THF. As a result, most reactions, had to be carried out in DMF. In addition, column-loading for silica gel flash chromatography was often difficult.

DOX and paclitaxel linker compounds **11** and **17** have been conjugated to the monoclonal antibody BR96 and the resulting immunoconjugates have shown excellent, immunospecific activities *in vitro*. Full biological results will appear in future publications.

In summary, we have shown the utility of MMT as an amino protecting group for the synthesis of compounds that are sensitive to a variety of deblocking conditions including those required to cleave most common acid-labile protecting groups. Deprotection can be carried out using reagents of "intermediate-level" acidity such as dichloro- and chloroacetic acids in CH_2Cl_2/anisole, thus avoiding inconvenient mixtures such as 80% acetic acid, with isolation of pure products involving simple precipitation and filtration.

References and Notes

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6. All new compounds gave satisfactory NMR, mass spectral, microanalytical and/or HRMS data.
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9. In a typical procedure a stirred solution of MC-Phe-Lys(MMT)-PABC-DOX **10** (1.152 g, 0.804 mmol) in CH_2Cl_2 (50 mL) at rt was treated with anisole (8.73 mL, 100 equiv.) and dichloroacetic acid (0.663 mL, 10 equiv.). After 1 h the mixture was diluted with ethyl acetate (200 mL). The resulting suspension was stored at 0°C for 1 h and then the orange solid product **11** was collected by filtration, washed repeatedly with ethyl acetate, and dried *in vacuo* (1.028 g, 99%). HPLC showed the product to be >99% pure. ^{1}H NMR ($CDCl_3/CD_3OD$) δ 1.10-1.90 (m, 14H), 1.21 (d, 3H), 2.10 (t, 2H), 2.20 (m, 2H), 2.88 (m, 2H), 3.02 (m, 2H), 3.12 (m, 2H), 3.38 (t, 2H), 3.52 (brs, 1H), 3.79 (m, 1H), 4.02 (s, 3H), 4.10 (m, 1H), 4.43 (m, 1H), 4.54 (m, 1H), 4.72 (s, 2H), 4.92 (m, 2H), 5.24 (brs, 1H), 5.44 (brs, 1H), 5.84 (s, 1H), 6.67 (s, 2H), 7.10 (brs, 5H), 7.21 and 7.48 (2 x d, each 2H), 7.38 (d, 1H), 7.77 (t, 1H), 7.99 (d, 1H). HPLC: (15 cm C-18 column, 4:1 CH_3OH/50 mM triethylammonium formate buffer (pH 2.8), 1 mL/min., λ = 495 nm): retention time: 4.5 min. MS (FAB$^-$): 1159 $(M-H)^-$. HRMS: Calc. for $C_{60}H_{68}N_6O_{18}Na$: 1183.4488. Found: 1183.4457.
10. A simple tritylamino-Lys version of **10** was prepared and subjected to the same deblocking conditions. After 1h less than 10% of **11** had formed.
11. For synthetic and structural details concerning MMC compounds, see the next communication in this issue.
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14. For **17**: ^{1}H-NMR ($CDCl_3$) δ 1.13, 1.20 and 1.72 (s, each 3H), 1.10-1.90 (m, 12H), 2.13 and 2.33 (s, each 3H), 2.96 (m, 2H), 3.05 (m, 2H), 3.38 (m, 2H), 3.86 (d, 1H), 4.21 (m, 2H), 4.50 and 4.61 (m, each 1H), 4.77 (brs, 1H), 4.91 (d, 1H), 5.10 (m, 2H), 5.42 (m, 1H), 5.64 (d, 1H), 5.71 (m, 1H), 5.89 (s, 1H), 6.11 (m, 1H), 6.30 (s, 1H), 6.73 (s, 2H), 7.00-8.20 (m, 24H), 9.23 (br, 1H). MS (FAB) 1471.6 $(MH)^+$, 1509.5 $(M+Na)^+$, 1511.8 $(M+K)^+$. HRMS: Calc for $C_{80}H_{89}N_6O_{21}$: 1470.6159. Found: 1470.6135.

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