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The Mitsunobu Reaction: A Novel Method for the Synthesis of Bifunctional Maleimide Linkers

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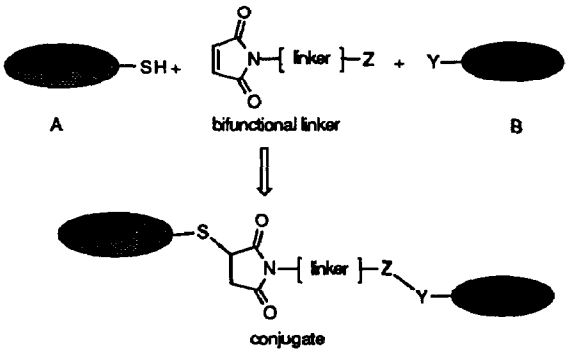
Abstract: Compounds **1-7** were synthesized from maleimide and the corresponding alcohols using a novel application of the Mitsunobu reaction. This procedure allows the direct formation of a variety of bifunctional linker compounds.

Bifunctional linkers, with a maleimido group attached to one end and a connectable functionality (eg. -OH, $-NH_2$, $-CO_2H$) on the opposite end, are useful for modifying proteins and peptides.[1] The maleimido group is one of a number of functionalities that are reactive with thiol nucleophiles,[2] thus allowing coupling of the linker to cysteine or -SH containing molecules (cf. scheme). The other molecule can be attached to the opposite end (Z) by means of an amide, ester, ether or other type of bond. These linkers are useful for synthesizing: peptide-conjugate haptens,[3] immobilized antibodies or enzymes[4], antibody-antibody conjugates[5], immuno-conjugates or -toxins,[6] enzyme inhibitors and enzyme probes.[7]

Scheme

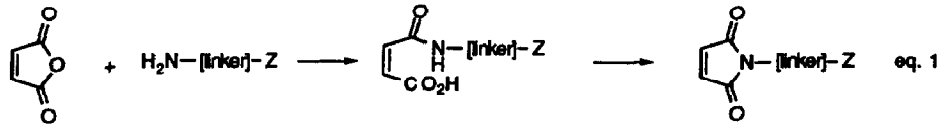


The standard method for synthesizing maleimido derivatives is shown in equation **1**. Typically, an amino-containing derivative is converted to an intermediate maleamic acid which is then cyclized under acidic or dehydrative conditions.[8] The yields are generally modest and the reaction fails to work with certain amines.[9] Another factor limiting the general applicability of this procedure is that the reaction conditions preclude the use of acid labile or other sensitive functionality.

Direct alkylation of maleimide would be a more convenient procedure. Only one example appears in the literature where maleimide is directly N-alkylated[10,11]. In this case, the corresponding silver and mercury salts were generated which in turn reacted with alkyl bromides, however, this reaction was only applied to the synthesis of simple N-alkyl derivatives.

The Mitsunobu reaction[12] shown in equation **2** is more general since it can be carried out under essentially neutral conditions and at room temperature. In addition to this, the starting material for this reaction is an alcohol rather than an amine allowing an alternative synthetic entry into this class of compounds. Surprisingly, an examination of the literature revealed that these conditions have not been used to synthesize N-substituted maleimides. Therefore, the method was tested to determine its utility in the synthesis of bifunctional linkers.

Literature Method:

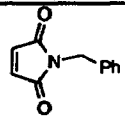
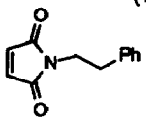
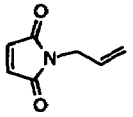
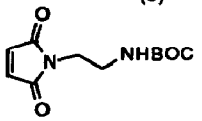
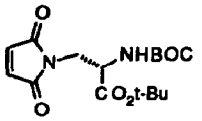
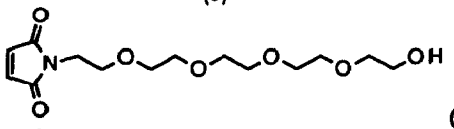
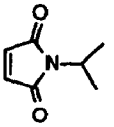


eq. 1

Mitsunobu Method:

eq. 2

Table. Synthesis of N-Alkylmaleimides Using the Mitsunobu Reaction

entry	N-substituted maleimide (#)	%yield (lit.)[a]	mp (°C)[b] (lit.)
1	 (1)	73(65)[c]	68-69(69-70)[c]
2	 (2)	52(73)[d]	110-111(112)[d]
3	 (3)	75(68)[c]	40-42(42-44)[c]
4	 (4)	57	125-127
5	 (5)	38[e]	100-102
6	 (6)	66[e]	oil
7	 (7)	31	oil(27)[f]

[a]Isolated yields based on maleimide. [b]Melting points are uncorrected. [c]Reference 8(e). [d]Reference 8(c). [e]2 equivalents of pentaethylene glycol per equivalent of maleimide were used in this reaction. [f]Reference 18.

As shown in the table, maleimide is well suited as a nucleophile in this reaction and it was successfully condensed with a variety of alcohols. Under typical conditions the alcohol was stirred overnight in THF with Ph_3P, DIAD[13] and maleimide

(1:1:1) to yield the N-alkylated maleimide following column chromatography (SiO_2).[14 15] The yields shown in the table are unoptimized and varied from 31-75%. The best yields were obtained for 1° alcohols adjacent to S_N2 activating groups(entries 1 and 3) where the yields exceeded those reported in the literature. With the exception of **5**,[16] other 1° alcohols gave modest yields (entries 2, 4 and 6). The lowest yield was obtained for the Mitsunobu reaction of isopropanol (entry 7). However, the synthesis N-*sec*-alkylmaleimides is also difficult using the literature methods.[17]

The described reaction thus represents a convenient and novel method for synthesizing complex maleimides. To the author's knowledge this appears to be the first example of the N-alkylation of maleimide using Mitsunobu conditions. The neutral conditions used in this procedure should allow the synthesis of more complex linkers. Research is currently under way to optimize the procedure and examine its utility with other alcohols.

Acknowledgments

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13. DIAD: diisopropylazidodicarboxylate.

14. The synthesis of **1** is given as a representative procedure. Benzyl alcohol (0.97 mL, 9.4 mmol) was dissolved in 75 mL of THF to which was added Ph_3P (2.66 g, 10.2 mmol), maleimide (1.00 g, 10.4 mmole) and DIAD (2.09 mL, 11.4 mmol). The resulting yellow solution was stoppered and stirred overnight. The solvent was then removed under reduced pressure and the resulting residue triturated with 1:1 hexanes/Et_2O to remove the Ph_3PO by-product. After removal of solvent the crude product was isolated by chromatography (SiO_2, 5:1 hexanes/EtOAc) to yield 1.28 g (73%) of **1** as a white solid. mp 68-69 °C. IR (KBr): 2948, 1700 cm^{-1}. ^1H NMR (300 MHz): δ 4.65 (s, 2), 6.68 (s, 2), 7.27 (m, 5). ^{13}C NMR (300 MHz): δ 41.40, 127.86, 128.38, 134.19, 136.19, 170.41. MS (CDI) 188 (MH^+).

15. Spectral data for compounds **2-7** are given below. **2**: ^1H NMR (300 MHz): δ 2.87 (dd, 2, J = 7.4), 3.74 (dd, 2, J = 7.3), 6.62 (s, 2), 7.24 (m, 5). ^{13}C NMR (300 MHz): δ 34.50, 39.09, 126.68, 128.56, 128.82. 134.02, 137.83, 170.55. MS (DCI): 202 (MH^+). **3**: ^1H NMR (300 MHz): δ 4.07 (m, 2), 5.13 (m, 2), 5.72 (m, 1), 6.67 (s, 2). ^{13}C NMR (300 MHz): δ 39.84, 117.55, 131.47, 134.16, 170.30. **4**: ^1H NMR (300 MHz): δ 1.35 (s, 9), 3.27 (q, 2, J = 6.0), 3.60 (dd, 2, J = 4.2, 6.0), 4.79 (br s, 1), 6.67 (s, 2). ^{13}C NMR (300 MHz): δ 28.28, 79.46, 134.15, 155.93, 170.82. MS (DCI) 241 (MH^+). **5**: ^1H NMR (300 MHz): δ 1.37 (s, 9), 1.44 (s, 9), 3.76 (dd, 1, J = 7.8, 14.0), 3.85 (dd, 1, J = 4.7, 14.0), 4.40 (m. 1), 5.24 (m, 1), 6.69 (s, 2). ^{13}C NMR (300 MHz): δ 27.90, 28.22, 39.53, 52.89, 79.95, 83.02, 134.19, 155.19, 168.81, 170.38. MS (DCI) 341 (MH^+). **6**: ^1H NMR (300 MHz): δ 2.82 (br s, 1), 3.53 (m, 20), 6.61 (s, 2). ^{13}C NMR (300 MHz): δ 37.06, 61.56, 67.70, 69.97, 70.26, 70.41, 70.50, 72.46, 134.10, 170.59. MS (DCI) 318 (MH^+). **7**: ^1H NMR (300 MHz): δ 1.32 (d, 6, J = 6.9), 4.28 (hept., 1, J = 6.9), 6.58 (s, 2). ^{13}C NMR (300 MHz): δ 19.99, 42.77, 133.84, 170.70.

16. Compound **5** was allowed to react with HCl (2 M in 1:1 CH_2Cl_2/dioxane) to remove the BOC protecting group. The resulting amino t-butyl ester was then acylated separately with the acid chloride of (+)-MPTA (α-methoxy-α-trifluoromethyl)phenylacetic acid) and (-)-MPTA which yielded the corresponding Mosher's amides. ^1H NMR analysis of these products showed an 80:20 mixture of diastereomers, indicating that the chiral center in **5** had been racemized slightly. A referee has suggested that an elimination/addition mechanism may account for this loss of stereochemical integrity. This might also be the reason for the low yield. For a reference to the dehydration of serine using Ph_3P/DEAD see: Wojciechowska, H.; Pawlosicz, R.; Andruszkiewcz, R.; Grzybowska, J. *Tetrahedron Lett.* **1978**, 4063.

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