Benzotriazole maleimide as a bifunctional reactant for SERS

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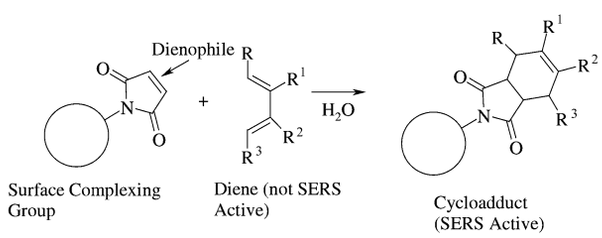
The synthesis of a benzotriazole maleimide is reported and its use as a bifunctional compound demonstrated. The triazole moiety of the compound complexes strongly with metals such as copper and silver and can be used to form monolayers on metal surfaces. The maleimide acts as a dienophile and reacts with dienes to produce cycloadducts. We report the selective reaction of the benzotriazole maleimide with seven different dienes to produce a range of cycloadducts. These cycloadducts were then adsorbed onto a metal surface *via* the triazole group. The presence on the metal surface was confirmed by surface enhanced Raman scattering, SERS. SERS is a vibrational spectroscopy and as such provides a fingerprint of each compound examined. The cycloadducts all gave different spectra that allowed identification of each diene that had cyclised. The dienes did not produce SERS on their own and had to be reacted with the bifunctional benzotriazole maleimide prior to examination. This provides an illustration of the use of bifunctional reactants specifically designed to produce SERS active products and also provides an example of an efficient derivatisation chemistry for copper and silver metal surfaces.

Introduction

Surface enhanced Raman scattering (SERS) is a vibrational spectroscopy that can provide molecularly specific information at ultra low concentrations.[1–3] SERS overcomes the two main drawbacks in using Raman scattering for sensitive analysis, in that great sensitivity is obtained and the problem of fluorescence is very much reduced.[4–6] The basic requirement of the technique is the adsorption of the analyte onto a suitable roughened metal surface, normally of silver or gold. The enhancement arises from a coupling of the radiation field to the surface plasmon which then couples to the polarisability of the analyte adsorbed on the metal surface, usually in the form of a deposited metal surface[7,8] or aqueous colloid.[9,10] In our studies we favour citrate reduced silver colloid which must be aggregated to provide areas of high electric field and hence maximum enhancement.[11] Aggregation of the colloid is achieved by using agents that alter the negative surface charge, such as sodium chloride or poly(L-lysine).[12,13] This is not a unique approach but it has provided excellent reproducibility and enhancement of the Raman scattering.

A major advantage of SERS as a detection technique is that most compounds will give rise to SERS once attached to the metal surface and, consequently, compared to other techniques such as fluorescence, a wider range of chemistries can be used to create suitable labels. SERS will not be observed until the analyte is adsorbed onto the metal surface. In this paper we show how we have developed a method of generating a specific SERS signal after a chemical reaction involving the analyte and a bifunctional SERS reactant. The bifunctional reactant has been designed to provide specific reactivity towards the analyte and to complex to the metal surface to provide SERS of the product.

We embarked on this project with a view to producing a novel labelling chemistry for use in biological analysis. Current detection techniques such as fluorescence tend to rely on large chemical moieties. These chemical moieties can then affect the biological activity of the system under investigation, reducing it to near zero in some cases either due to steric effects or the ability of the label to be biologically active itself.[14,15] Here we use small inert molecules that act as tags and are not visible by SERS until exposed to the bifunctional SERS reactant (Scheme 1). The reaction we have chosen to use for the conversion of the tag into a SERS active species is a Diels–Alder cycloaddition. Diels–Alder cycloadditions are accelerated in aqueous solution which makes them compatible with our colloidal system.[16–20] Additionally, dienes are four-carbon units which are small and relatively inert towards any biological nucleophiles. To be used as tags they can be easily added by conventional methods to molecules of interest such as DNA.



Scheme 1 Use of a bifunctional reactant to generate a SERS active species under aqueous conditions.

The bifunctional reactant must be a dienophile that complexes strongly to a silver surface. We have chosen benzotriazole as the complexing group and added a maleimide function to act as the dienophile. Previously we have synthesised a range of model compounds for use in SERS studies that were based on a benzotriazole (BTH) moiety.[21] The BTH complexes very strongly with metals and has been used as an anti-tarnish agent for many years.[22] It is believed to form a polymeric species with silver ions on the surface and we now have evidence that the benzotriazole displaces the surface layer of citrate found on the particles of silver colloid normally used.[23] Thus, by incorporating a benzotriazole moiety into a compound, strong essentially irreversible surface complexation is assured. Systems based on this philosophy have proved in practice to be suitable for semi-quantitative or quantitative analysis.[24]

In this study we show how a benzotriazole dienophile was synthesised and then reacted with a variety of dienes in aqueous solution to produce SERS active cycloadducts with spectra specific to each diene used. This has implications for the tagging of molecules of interest that are not SERS active with dienes to selectively make them SERS active. Although the study was specifically designed to further improve

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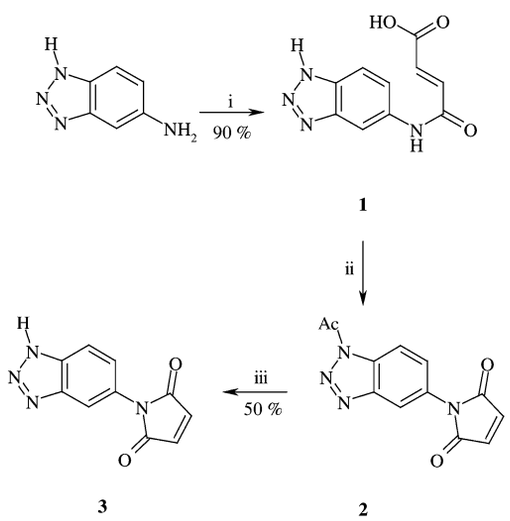
the advantages of SERS for biological detection, it also provides a very attractive route to modify and functionalise silver surfaces. It is potentially useful for the immobilisation of biological ligands. Among the advantages are, the small facile nature of the tag to the analyte, the facile nature of the attachment chemistry, and the effective nature of the BTH attachment. A specific advantage of the procedure is that SERS can be used to monitor the reaction *in situ*, at least for roughened silver nanoparticle surfaces.

Results and discussion

The dienophile synthesised for this study was a benzotriazole maleimide (BTM). A range of dienes such as butadienes and furans were examined as potential labelling agents. These dienes form the small tags which after cycloaddition with the maleimide produce a SERS active product where the benzotriazole promotes robust surface adhesion as discussed above.

Benzotriazole dienophile

The BTM was synthesised in a three-step procedure. In the first step maleic anhydride was dissolved in dichloromethane and added to 5-aminobenzotriazole in acetone to produce 3-(1*H*-benzotriazol-5-ylcarbamoyl)acrylic acid (**1**) (Scheme. 2). This



Scheme 2 Synthesis of the bifunctional reactant. *Reagents and conditions:* (i) maleic anhydride, CH_2Cl_2–acetone, RT, 4 h; (ii) acetic anhydride, 90 °C; (iii) TFA, 12 h, RT.

acrylic acid analogue was then refluxed in acetic anhydride to form 1-(1*H*-benzotriazol-5-yl)pyrrole-2,5-dione (**3**) (BTM). However, in addition to the ring formation, partial acetylation of the triazole ring occurred during this step. A mixture of the 5- and 6-isomers of the acetylated benzotriazole moiety were obtained due to tautomerism of the triazole function, although the 5-isomer (**2**) of the BTM was the major product. The isomers were not isolated and were used as a mixture for the deprotection of the triazole ring. This was carried out in quantitative yield using trifluoroacetic acid to give the pure product in an overall yield of 45%. The BTM was then used as the dienophile in all the following Diels–Alder cycloadditions.

Diels–Alder reactions of the benzotriazole maleimide

Several aqueous based Diels–Alder reactions were successfully attempted with BTM (Scheme 3) and suitable dienes. Reactions were attempted in water with acetonitrile as a co-solvent since this increases the rate of Diels–Alder reactions due to the hydrophobic packing effect, which promotes aggregation of non-polar species in an aqueous environment. In some cases lithium chloride was also used, as it is known to increase the hydrophobic interaction of organic molecules.[16–18,20] Similarly copper(II) nitrate was used in some cases as a catalyst to enhance the rate of reaction of the diene with the BTM. Lewis acids such as copper(II) nitrate increase the reactivity of BTM towards cycloaddition by forming a complex with the two carbonyl functional groups and hence significantly lower the energy level of the LUMO.[25,26]

The first diene used was 9-anthracene carbinol as its use in Diels–Alder reactions in water is well documented.[17,18] We discovered that reactions of this nature are unsuccessful when carried out with our compounds in water alone, but went to completion with the addition of small amount of acetonitrile as a co-solvent. The very low aqueous solubility of both starting materials appears to be the limiting factor since Diels–Alder reactions in water are successful only for compounds with a minimum degree of solubility. Also, due to the high concentration of lithium chloride, acetonitrile was not miscible with water and though the two starting materials were both in the organic phase, the reaction was believed to occur at the interface of the two phases.[17] Once this had been determined the product 2-(1*H*-benzotriazol-5-yl)-4,9-[1′,2′]benzeno-4-(hydroxymethyl)-3a,4,9,9a-tetrahydro-1*H*-benz[*f*]isoindole-1,3(2*H*)-dione (**4**) was easily synthesised in good yield.

Three buta-1,3-diene analogues were used to successfully react with the BTM. All of the butadienes had electron-donating groups as this promotes the cycloaddition reaction, and in addition one had an electron-withdrawing group. 2,3-Dimethylbuta-1,3-diene reacted with the BTM to produce 2-(1*H*-benzotriazol-5-yl)-5,6-dimethyl-3a,4,7,7a-tetrahydroisoindole-1,3-dione (**5**) in excellent yield. Similarly 1-methoxybuta-1,3-diene reacted cleanly with the BTM to produce 2-(1*H*-benzotriazol-5-yl)-4-methoxy-3a,4,7,7a-tetrahydroisoindole-1,3-dione (**6**). Hexa-2,4-dienoic acid produced the expected 2-(1*H*-benzotriazol-5-yl)-7-methyl-1,3-dioxo-2,3,3a,4,7,7a-hexahydro-1*H*-isoindole-4-carboxylic acid (**7**), but in lower yield than the previous two butadienes. This was attributed to the presence of the carboxylic acid functionality which had an electron withdrawing effect on the butadiene, making the reaction less favoured compared to the other two butadienes. In addition, this compound was harder to isolate compared to the previous compounds which reduced the yield further. The cycloadditions of the butadienes were all carried out using an acetonitrile–water mix with 4.86 M lithium chloride to increase the rate of reaction. The cycloadditions occurred in an acetonitrile–water mix alone but took longer to reach completion, thus justifying the use of lithium chloride.

Diels–Alder reactions with furan and 2,5-dimethylfuran were also investigated. The dienes were dissolved in an acetonitrile–water mixture and 0.01 M copper(II) nitrate added as a Lewis acid catalyst. In contrast to the previous reactions involving the butadienes and anthracene carbinol, no reaction occurred unless a catalyst was employed. Furan itself reacted cleanly with the BTM under the above conditions to give 4-(1*H*-benzotriazol-5-yl)-10-oxa-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione (**8**) and 2,5-dimethylfuran produced 4-(1*H*-benzotriazol-5-yl)-1,7-dimethyl-10-oxa-4-azatricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione (**9**). The cycloaddition involving the dimethylfuran is higher yielding and can be explained by the increased electron density of the diene system. A mixture of *endo* and *exo* isomers was obtained in a 2 : 1 ratio for both reactions involving the furans. The major isomer was assumed to be the *endo* as this is usually the major adduct formed due to the Alder's Rule. The *endo* cycloadduct is also favoured when water is used as a solvent.[17,18] The 2 : 1 mixture of *endo* and *exo* isomers was used in the SERS studies without separation.

Cycloaddition with furan-2-carbaldehyde dimethylhydrazone 30 minutes after mixing with BTM in an acetonitrile–water mixture was more unusual in that an aromatic ring

system was created as a result of spontaneous dehydration following the cycloaddition.[27] 2-(1*H*-Benzotriazol-5-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-4-carbaldehyde dimethylhydrazone (**10**) was produced as a precipitate, which was easily isolated. Spontaneous loss of water occurred due to the electron-donating group on the furan ring to produce a highly conjugated system.

SERS of the Diels–Alder cycloadducts

SERS spectra were obtained by adding the appropriate cycloadduct to a suspension of silver colloid. Citrate reduced silver colloid has a protective layer of citrate on the surface of the silver and as such is negatively charged. The benzotriazole displaces the citrate from the surface and complexes directly with the surface.[27] This brings the part of the molecule formed by the cycloaddition reaction to within the minimum distance from the surface to experience surface enhancement. Sodium chloride was then added to aggregate the individual colloidal particles into discreet aggregates. This creates extremely high electric fields at the interstices in the aggregate. This procedure averages the enhancement from indiviual particles to give a degree of control over the process and a superior signal to noise ratio.

SERS of the cycloadducts using an excitation wavelength of 457.9 nm are shown in Fig. 1 for the butadienes and Fig. 2 for the furans. Aggregation created by sodium chloride is carried out in such a way as to maintain the colloid particles in suspension. The dienes themselves did not produce SERS except for the furan-2-carbaldehyde dimethylhydrazone which has a positive amine function that allows surface adsorption. This resulted in some signals being produced, although these were very weak and different from the cyclcoadduct.

The benzotriazole maleimide produces a spectrum with five main bands. The carbonyl stretching vibration gave a band at 1766 cm^{-1}. The band at 1611 cm^{-1} was assigned to a stretch of the phenyl ring and the band at 1385 cm^{-1} to a stretch of the triazole ring. The remaining two bands at 1170 and 1096 cm^{-1} were assigned to stretches of the benzotriazole ring system. These five bands were the main features for all the compounds. However, there were several differences which could be used to discriminate between the different compounds. The major difference between BTM and the cycloadducts was a change in intensity between the bands around 1440 and 1390 cm^{-1}. For the maleimide, the band at 1422 cm^{-1} was weak and obscured by the stronger scattering from the band at 1385 cm^{-1}. However for the Diels–Alder adducts, the band around 1420 cm^{-1} was

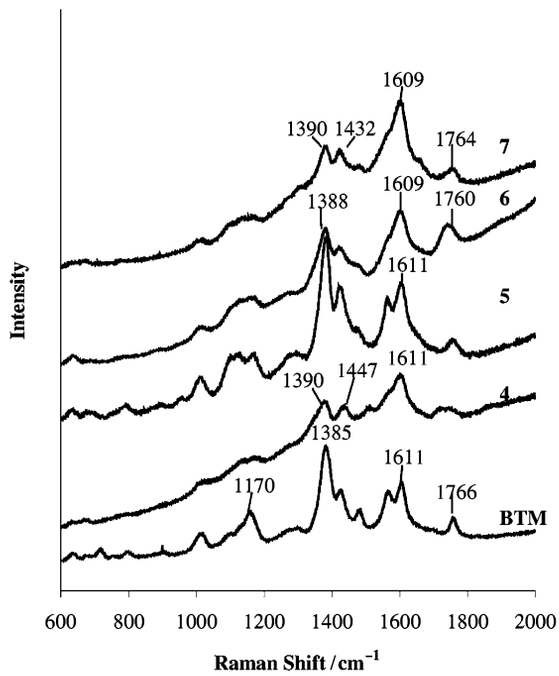
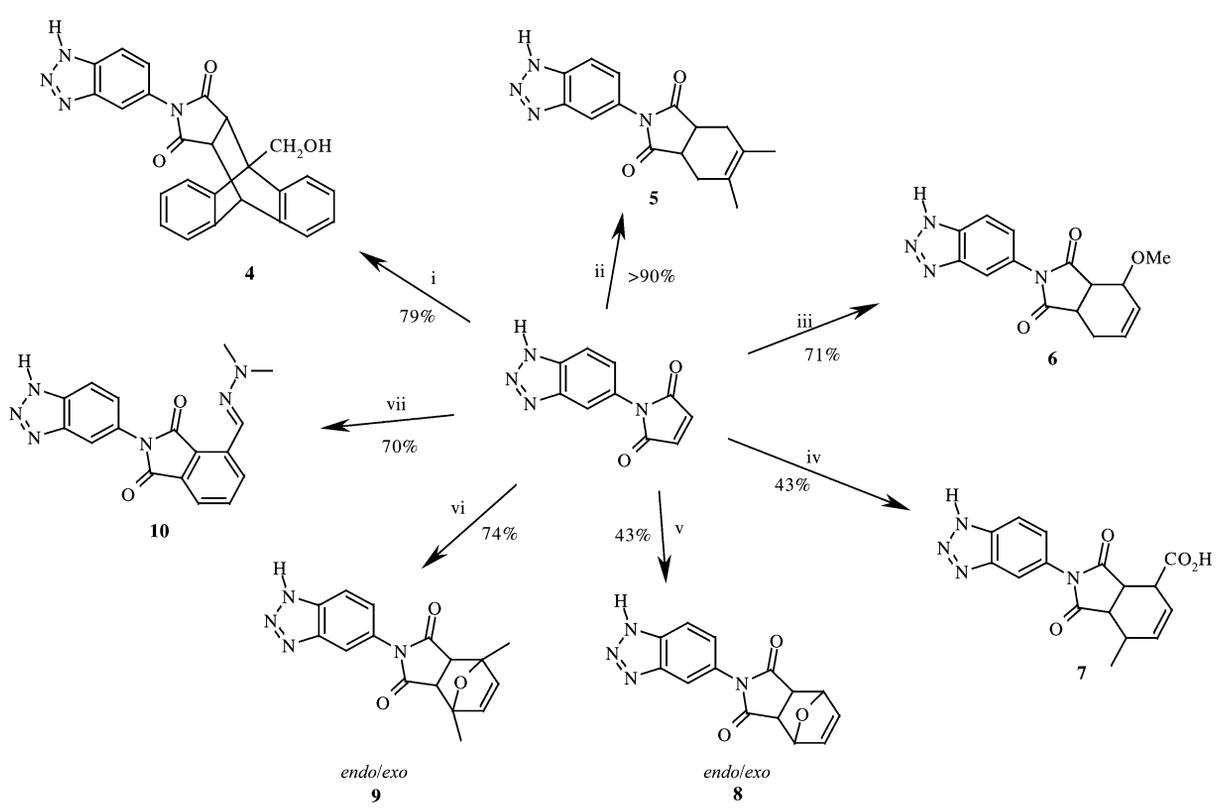


Fig. 1 SERS spectra of the cycloadducts produced from anthracene carbinol and the butadienes at approximately 3×10^{-8} moles in 400 μl using one accumulation of 10 seconds.



Scheme 3 Synthesis of the cycloadducts from BTM and the corresponding dienes. *Reagents and conditions:* (i), (ii), (iii), (iv) H_2O–CH_3CN, LiCl 4.86 M, 45 °C, 12 h; (v) H_2O–CH_3CN, $Cu(NO_3)_2$, 0.01 M, RT, 12 h; (vi) H_2O–CH_3CN, $Cu(NO_3)_2$ 0.01 M, 45 °C 12 h; (vii) H_2O–CH_3CN, 45 °C, 0.5 h.

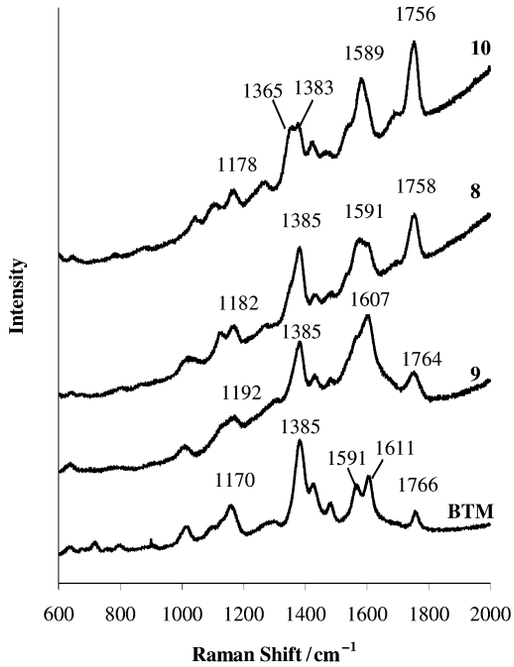


Fig. 2 SERS spectra of the cycloadducts produced from the furans at approximately 3×10^{-8} moles in 400 μl using one accumulation of 10 seconds.

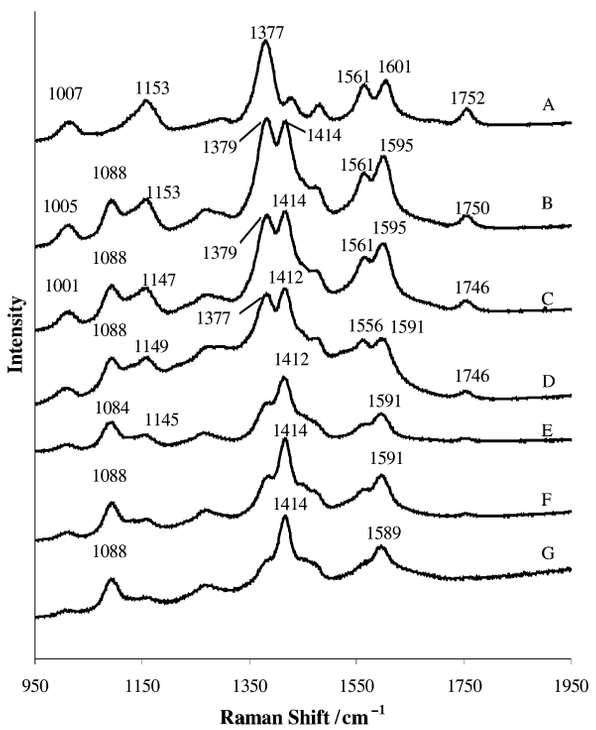


Fig. 3 SERS spectra of BTM at decreasing concentrations using three 10 second accumulations of a 1 ml suspension. A = 1×10^{-8} moles, B = 1×10^{-9} moles, C = 8×10^{-10} moles, D = 6×10^{-10} moles, E = 4×10^{-10} moles, F = 2×10^{-10} moles, G = 1×10^{-10} moles.

clearly observed. Further, the peak at 1096 cm^{-1} lost most of its intensity in the spectra of the Diels–Alder adducts.

A study of the effect of concentration on the SERS of BTM showed that at concentrations between 1×10^{-8} and 1×10^{-10} moles in 1 ml of colloid there were changes in the relative intensities of the peaks. The results are shown in Fig. 3.

The major differences were that the peaks at 1752, 1601, 1473, 1377, 1153 and 1007 cm^{-1} in the 1×10^{-8} mole spectrum disappeared as the concentration was reduced and peaks at 1414 and 1088 cm^{-1} appeared strongly in the 1×10^{-10} mole spectrum. These results indicate the presence of two adsorbed

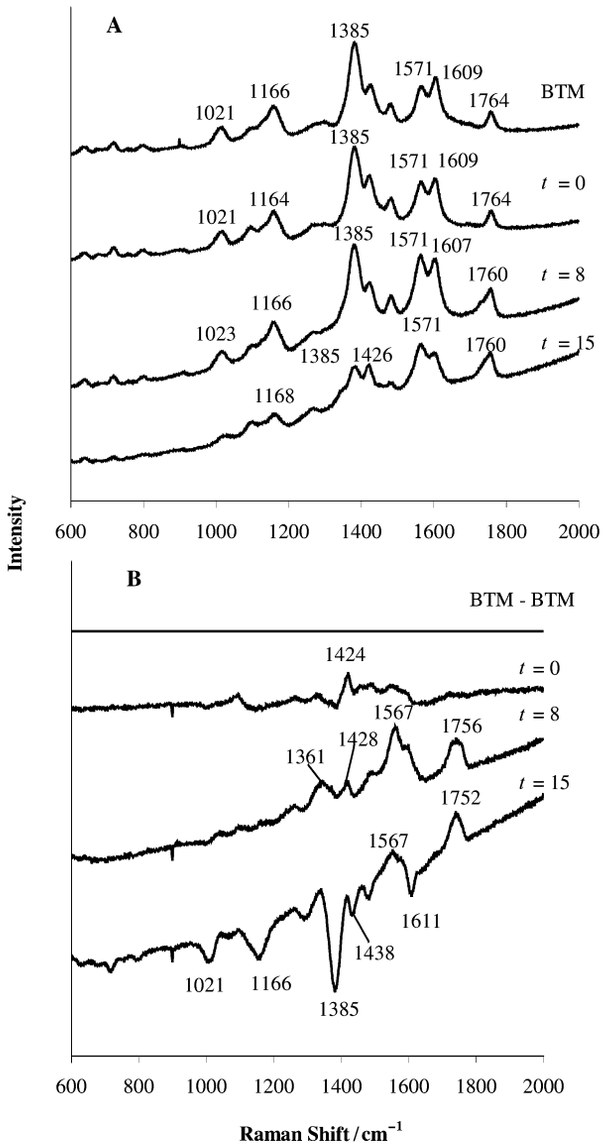


Fig. 4 **A** – SERS spectra of furan-2-carbaldehyde dimethylhydrazone mixed with BTM at 1×10^{-8} moles in 1 ml and monitored over regular time intervals. Three accumulations of 10 seconds were used and time is in minutes. **B** – Difference spectra of the reaction mixture (minus BTM).

forms of BTM. The carbonyl stretch is more intense in the spectrum of the predominant species at low concentration and the triazole ring stretch is more intense in the spectrum at high concentration. SERS signals are dependent on the orientation of the molecule relative to the silver surface where vibrations with large polarisability changes perpendicular to the surface produce enhanced intensity. The changes observed suggest that the planar ring system lies flatter on the surface at low concentrations. The change in triazole frequencies suggests a different bonding mode in each case. The BTM is expected to form a silver complex on the surface where the form of the complex and hence the orientation may change as surface coverage approaches saturation to alter the thermodynamically favoured mode of complexing.

The cycloaddition reaction can also be followed over time by SERS as shown in Fig. 4A. Again the signals differ for different concentrations, although they are consistent with the formation of the cycloadduct (**10**). Discrimination between the product and BTM is based upon the differences in the spectrum. A series of difference spectra are also shown (Fig. 4B) where the spectrum of BTM has been subtracted from the spectrum of the reaction mixture at the same time intervals. At 1×10^{-8}

moles the major differences between the BTM and **10** are the loss in intensity of the peaks at 1021, 1166, 1385 and 1611 cm^{-1} and the gain in intensity of the peaks at 1567 and 1752 cm^{-1}. The difference spectra clearly show the change in the reaction mixture over time, with the changes becoming more distinct with time. Thus we can use this method to accurately identify the reaction of the benzotriazole maleimide with the dienes.

Conclusion

In conclusion we have synthesised a benzotriazole maleimide which is a bifunctional reactant that acts selectively in a Diels–Alder cycloaddition and then complexes to a metal surface. This bifunctional reactant was successfully used as a dienophile in seven Diels–Alder reactions to produce a range of different cycloadducts. The cycloadducts were then examined by SERS using citrate reduced silver colloid as the metal surface for enhancement. Good SERS signals were obtained for all of the cycloadducts due to the presence of the benzotriazole group. Each spectrum was distinct and related to the individual molecular structure of the cycloadduct, although changes in the spectra were observed to be related to concentration. This allows quantitation of the material present by using the differences in the spectra as opposed to absolute intensities. We have developed a type of chemistry that allows us to produce distinct SERS signals from a class of small molecules that do not normally provide good SERS. It is envisaged that these small molecules can be used as tags for molecules of interest that become active after cycloaddition as described in this study. This is a totally novel approach to SERS labelling and will prove invaluable for future SERS based detection systems.

Experimental

All chemicals were purchased from Aldrich Ltd except 5-aminobenzotriazole (Lancaster Synthesis Ltd). ^{1}H NMR spectra were recorded on a Bruker 400 (400 MHz) instrument. Mass spectra were recorded on a JEOL AX505 spectrometer using electron impact ionization (EI) at 70 eV or fast atom bombardment (FAB) using a 3-nitrobenzyl alcohol matrix. Flash chromatography was carried out using silica gel 60 (Merck). Thin layer chromatography was carried out on aluminium sheets, silica gel 60 F_{254}, 0.2 mm layer (Merck) (A) EtOAc–MeOH–NH_4OH (5 : 1 : 1); (B) DCM–MeOH 9 : 1; (C) EtOAc–hexane (7 : 3); (D) EtOAc–hexane (8 : 2). SERS spectra were recorded with a Renishaw 2000 Raman Microprobe instrument with 457.9 nm excitation provided by a Spectra-Physics Model 2020 argon-ion laser (100 mW).

Chemical synthesis

3-(1*H*-Benzotriazol-5-ylcarbamoyl)acrylic acid (1). Maleic anhydride (1.20 g, 12 mmol) was dissolved in dichloromethane (30 ml) and 5-aminobenzotriazole (0.91 g, 7 mmol) in acetone (20 ml) added dropwise. After stirring at room temperature for 4 h TLC showed complete reaction. The product was isolated by filtration, washed with acetone and then dried to yield the title compound as a grey powder (1.50 g, 93%); mp 212–214 °C; R_f (A) 0.0; $\delta_H((CD_3)_2SO)$: 6.35 (1H, d, *J* 12.0, C*H*CHCO$_2$H), 6.53 (1H, d, *J* 12.0, CHC*H*CO$_2$H), 7.41 (1H, br s, H-7), 7.91 (1H, br s, H-4), 8.36 (1H, br s, H-6), 10.6 (1H, s, CO$_2$*H*), 12.97 (1H, br s, N*H*CO), 15.5 (1H, br s, NH); *m*/*z* (EI) 232.05988 [$C_{10}H_8N_4O_3$ (M^+) < 1 ppm]; λ_{max}(DMSO–H_2O)/nm 244 (ε/dm^3 mol^{-1} cm^{-1} 9600), 294 (ε/dm^3 mol^{-1} cm^{-1} 8900).

1-(1-Acetyl-1*H*-benzotriazol-5-yl)pyrrole-2,5-dione (2). Anhydrous sodium acetate (1.72 g, 21 mmol) was dissolved in acetic anhydride (160 ml) and compound **1** (3.24 g, 14 mmol) added portionwise before heating the mixture at 90 °C for 2 h. After removal of the acetic anhydride *in vacuo*, the residue was washed with water, then pet. ether (40–60) before being dissolved in chloroform and dried over anhydrous Na_2SO_4. Removal of the solvent afforded 1-(1-acetyl-1*H*-benzotriazol-5-yl)pyrrole-2,5-dione with traces of 1-(1-acetyl-1*H*-benzotriazol-6-yl)pyrrole-2,5-dione (3.13 g, 87%); R_f (B) 0.71; *m*/*z* (EI) 256.05948 [$C_{12}H_8N_4O_3$ (M^+) < 0.6 ppm]; 1-(1-acetyl-1*H*-benzotriazol-5-yl)pyrrole-2,5-dione: $\delta_H(CDCl_3)$: 3 (3H, s, CH_3), 6.94 (2H, s, C*H*C*H*), 7.70 (1H, d, *J* 8.8, H-7); 8.17 (1H, s, H-4), 8.39 (1H, d, *J* 8.8, H-6); 1-(1-acetyl-1*H*-benzotriazol-6-yl)-pyrrole-2,5-dione: (3.13 g, 87%); $\delta_H(CDCl_3)$: 3 (3H, s, CH_3), 6.94 (2H, s, C*H*C*H*), 7.56 (1H, d, *J* 8.8, H-7); 8.23 (1H, d, *J* 8.8, H-6), 8.34 (1H, s, H-4).

1-(1*H*-Benzotriazol-5-yl)pyrrole-2,5-dione (BTM) (3). 1-(1-Acetyl-1*H*-benzotriazol-5-yl)pyrrole-2,5-dione (6.72 g, 26 mmol) was dissolved in TFA (130 ml) and the mixture was left to stir at RT overnight. After removal of TFA *in vacuo*, cold water was added and the precipitate collected by filtration, washed with pet. ether (40–60) and dried. The product was purified by column chromatography eluting with EtOAc in hexane (10–70%) to yield the title compound as a yellow powder (3.09 g, 55%); mp 107 °C (decomp.) (Found: C, 56.17; H, 3.19; N, 23.79%. $C_{10}H_6N_4O_2$ requires: C, 56.07; H, 2.80; N, 26.17%); R_f (B) 0.18; $\delta_H((CD_3)_2SO)$: 7.24 (2H, s, C*H*=C*H*), 7.42 (1H, d, *J* 8.8, H-7), 7.92 (1H, s, H-4), 8.02 (1H, d, *J* 8.0, H-6); *m*/*z* (EI) 214.04968 [$C_{10}H_6N_4O_2$ (M^+) < 2.8 ppm]; λ_{max}(DMSO–H_2O)/nm 262 (ε/dm^3 mol^{-1} cm^{-1} 5600), 279 (ε/dm^3 mol^{-1} cm^{-1} 5400).

2-(1*H*-Benzotriazol-5-yl)-4,9-[1′,2′]benzeno-4-(hydroxymethyl)-3a,4,9,9a-tetrahydro-1*H*-benz[*f*]isoindole-1,3(2*H*)-dione (4). Anthracene-9-methanol (50 mg, 0.24 mmol) and BTM (20 mg, 0.09 mmol) were dissolved in acetonitrile (15 ml) and added to an aqueous solution of LiCl (25 ml, 4.86 M) and the reaction left to stir overnight at 45 °C. The organic phase was collected and the aqueous phase extracted with DCM (2 × 10 ml). The combined extracts were dried and the solvent removed *in vacuo*. The resulting precipitate was washed with Et_2O to yield the title compound as a white powder (30 mg, 79%); mp 245 °C (decomp.) (Found: C, 63.72; H, 4.76; N, 12.03%. $C_{25}H_{18}N_4O_3 \cdot \frac{8}{3}H_2O$ requires: C, 63.83; H, 4.96; N, 11.91%). R_f (C) 0.21; $\delta_H((CD_3)_2SO)$: 3.48 (2H, s, CH_2OH), 4.88 (2H, s, 2 × (COC*H*)), 6.41 (1H, d, *J* 8.7, COCHC*H*), 6.98 (1H, s, H-4), 7.17–7.35 (7H, m, Ar-H), 7.46 (1H, d, *J* 7.2, H-7), 7.51 (1H, d, *J* 7.3, H-6), 7.86 (1H, d, *J* 8.7, Ar-H); *m*/*z* (EI) 422.14150 [$C_{25}H_{18}N_4O_3$ (M^+) < 8.5 ppm]; λ_{max}(DMSO–H_2O)/nm 255 (ε/dm^3 mol^{-1} cm^{-1} 10300), 271 (ε/dm^3 mol^{-1} cm^{-1} 8900).

2-(1*H*-Benzotriazol-5-yl)-5,6-dimethyl-3a,4,7,7a-tetrahydroisoindole-1,3-dione (5). Following the same procedure as for the synthesis of **4**, 2,3-dimethylbuta-1,3-diene (23 mg, 0.28 mmol) and BTM (30 mg, 0.14 mmol) afforded the title compound as a white powder in a quantitative yield (38 mg, 93%); mp 150 °C (decomp.) (Found: C, 62.43; H, 5.23; N, 18.41%. $C_{25}H_{18}N_4O_3 \cdot \frac{2}{3}H_2O$ requires: C, 62.34; H, 5.63; N, 18.18%); R_f (D) 0.34; $\delta_H((CD_3)_2SO)$: 1.69 (6H, s, 2 × CH_3), 2.37 (4H, s, 2 × (COCH_2)), 3.28 (2H, s, 2 × (C*H*CO)), 7.18 (1H, br s, H-7), 7.6–8.2 (2H, m, H-4, H-6); *m*/*z* (EI) 296.12616 [$C_{16}H_{16}N_4O_2$ (M^+) < 3.9 ppm]; λ_{max}(CH_3CN)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 6500), 280 (ε/dm^3 mol^{-1} cm^{-1} 5600).

2-(1*H*-Benzotriazol-5-yl)-4-methoxy-3a,7,7a-tetrahydroisoindole-1,3-dione (6). Following the same procedure as for the synthesis of **4**, 1-methoxybuta-1,3-diene (23 mg, 0.28 mmol) and BTM (30 mg, 0.14 mmol) afforded the title compound as a white powder (30 mg, 71%); mp 60 °C (decomp.); R_f (C) 0.23; δ_H(acetone-d_6): 2.60 (2H, m, CH_2CO), 3.32 (4H, m, COC*H*, OCH_3), 3.48 (1H, m, COC*H*), 4.33 (1H, m, C*H*COCH$_3$), 6.22 (2H, m, C*H*=C*H*), 7.33 (1H, br s, H-7), 7.87–8.04 (2H, m, H-4, H-6), 15.2 (1H, br s, NH); *m*/*z* (EI) 298.10707 [$C_{15}H_{14}N_4O_3$

(M^+) < 1.6 ppm]; λ_{max}(CH_3CN)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 5400), 281 (ε/dm^3 mol^{-1} cm^{-1} 4600).

2-(1*H*-Benzotriazol-5-yl)-7-methyl-1,3-dioxo-2,3,3a,4,7,7a-hexahydro-1*H*-isoindole-4-carboxylic acid (7). Following the same procedure as for the synthesis of **4**, hexa-2,4-dienoic acid (31 mg, 0.28 mmol) and BTM (30 mg, 0.14 mmol) afforded the title compound as a slightly yellow powder (20 mg, 43%); mp 223 °C (decomp.); R_f (C) 0.0; δ_H(CD_3OD): 1.45 (3H, d, *J* 7.4, C*H*$_3$), 2.57 (1H, m, C*H*CH$_3$), 3.32 (2H, m, 2 × (COC*H*)), 3.91 (1H, m, C*H*CO$_2$H), 5.81 (1H, m, C*H*=CHCHCO$_2$H), 6.53 (1H, m, CH=C*H*CHCO$_2$H), 7.03 (1H, d, *J* 8.7, H-7), 7.63 (1H, s, H-4), 7.84 (1H, d, *J* 8.6, H-6); *m*/*z* (FAB) 327.10967 [$C_{16}H_{15}N_4O_4$ (M + H)$^+$ < 1 ppm]; λ_{max}(DMSO–H_2O)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 3300).

***endo*,*exo*-4-(1*H*-Benzotriazol-5-yl)-10-oxa-4-azatricyclo-[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (8).** Furan (19 mg, 0.28 mmol) and BTM (30 mg, 0.14 mmol) were dissolved in acetonitrile (15 ml) and this solution added to an aqueous solution of $Cu(NO_3)_2$ (25 ml, 0.01 M). The reaction was left to stir overnight at RT by which time TLC showed complete reaction. The organic phase was collected and the aqueous phase extracted with DCM (2 × 10 ml). The combined extracts were dried and the solvent removed *in vacuo* to yield a mixture of the two isomers as a white powder (17 mg, 43%) with a 2 : 1 ratio of *endo–exo*; *m*/*z* (FAB) 283.08116 [$C_{14}H_{11}N_4O_3$ (M + H)$^+$ < 6.9 ppm]; λ_{max}(CH_3CN)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 5900), 281 (ε/dm^3 mol^{-1} cm^{-1} 5200). The two isomers were partially isolated by flash column chromatography eluting with EtOAc in hexane (0–70%); *endo*: R_f (EtOAc) 0.33; δ_H(acetone-d_6): 3.14 (2H, s, 2 × (COC*H*)), 5.30 (2H, m, 2 × (OC*H*)), 6.66 (2H, m, C*H*=C*H*), 7.35 (1H, d, *J* 8.8, H-7), 7.85 (1H, s, H-4), 8.00 (1H, d, *J* 8.8, H-6); *exo*: R_f (EtOAc) 0.40; δ_H(acetone-d_6): 3.74 (2H, s, 2 × (COC*H*)), 5.40 (2H, m, 2 × (OC*H*)), 6.67 (2H, m, C*H*=C*H*), 7.25 (1H, d, *J* 8.8, H-7), 7.74 (1H, s, H-4), 7.96 (1H, d, *J* 8.6, H-6).

***endo*,*exo*-4-(1*H*-Benzotriazol-5-yl)-1,7-dimethyl-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (9).** Following the same procedure as for the synthesis of **8** except that the reaction was carried out at 45 °C instead of RT, 2,5-dimethylfuran (27 mg, 0.28 mmol) and BTM (30 mg, 0.14 mmol) yielded a mixture of the two isomers as a white powder (32 mg, 74%) with a 2 : 1 ratio of *endo–exo*; R_f (D) 0.14; *endo*: δ_H(acetone-d_6): 1.69 (6H, s, 2 × C*H*$_3$), 3.13 (2H, s, 2 × (COC*H*)), 6.46 (2H, m, C*H*=C*H*), 7.36 (1H, d, *J* 8.8, H-7), 7.86 (1H, s, H-4), 8.00 (1H, d, *J* 8.7, H-6), 14.8 (1H, br s, N*H*); *exo*: δ_H(acetone-d_6): 1.76 (6H, s, 2 × C*H*$_3$), 3.47 (2H, s, 2 × (COC*H*)), 6.50 (2H, m, C*H*=C*H*), 7.26 (1H, d, *J* 8.8, H-7), 7.75 (1H, s, H-4), 7.95 (1H, d, *J* 8.5, H-6), 14.8 (1H, br s, N*H*); *m*/*z* (FAB) 311.11479 [$C_{16}H_{15}N_4O_3$ (M + H)$^+$ < 1.2 ppm]; λ_{max}(CH_3CN)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 5200), 281 (ε/dm^3 mol^{-1} cm^{-1} 5300).

2-(1*H*-Benzotriazol-5-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindole-4-carbaldehyde dimethylhydrazone (10). Furan-2-carbaldehyde dimethylhydrazone (39 mg, 0.28 mmol) and BTM (30 mg, 0.14 mmol) were dissolved in acetonitrile (15 ml) and added to distilled water (25 ml). The reaction was left to stir for 0.5 h at 45 °C by which time TLC showed complete reaction. The precipitate in suspension was collected, washed with Et_2O and dried to yield the title compound as an orange powder (33 mg, 70%); mp 228 °C (decomp.); R_f (EtOAc) 0.55; δ_H($(CD_3)_2SO$): 3.09 (6H, s, 2 × C*H*$_3$), 7.53 (1H, s, C*H*=N), 7.77 (2H, m, Ar-H), 8.04 (2H, s, Ar-H), 8.22 (1H, d, *J* 8.8, H-6), 15.95 (1H, br s, N*H*); *m*/*z* (EI) 334.11735 [$C_{17}H_{14}N_6O_2$ (M^+) < 1.4 ppm]; λ_{max}(DMSO–H_2O)/nm 245 (ε/dm^3 mol^{-1} cm^{-1} 13500), 320 (ε/dm^3 mol^{-1} cm^{-1} 7400).

Colloid preparation

Colloidal silver suspensions were prepared according to the Lee and Meisel[28] procedure with the conditions specified by Munro *et al*.[11]

SERS measurements

A stock solution of each compound (1 mg in 1 ml) was made up in DMSO for BTM 4.6 mM, (**4**) 2.4 mM, (**7**) 3 mM, (**10**) 3 mM and in CH_3CN for (**5**) 3.4 mM, (**6**) 3.4 mM, (**8**) 3.5 mM, (**9**) 3.2 mM. All solutions were diluted by a factor of ten in distilled water. Those solutions (50 μl) were then added to silver colloid (300 μl). NaCl (50 μl, 0.5 M) was used as an aggregating agent. The final concentrations for each compound were: BTM 58 μM, (**4**) 30 μM, (**5**) 42 μM, (**6**) 42 μM, (**7**) 38 μM, (**8**) 44 μM, (**9**) 40 μM, (**10**) 37 μM.

Raman scattering was collected using a Renishaw 2000 spectrometer and a 25 mW Spectra Physics Argon ion laser which delivered approximately 3 mW at the sample. The solutions were excited in capillary tubes using a Ventacom macrosampler or in a 96 well microtitre plate with a ×20 microscope objective. Individual scan times are found in the figure captions. The solutions for examination of BTM and BTM mixed with the furan-2-carbaldehyde dimethylhydrazone were made up of 1 ml of colloid, 50 μl of sample and 50 μl of NaCl. Spectra were offset by an arbitrary value to enable visualisation on the same chart.

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