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Synthesis of a Bicyclic Transition-State Analogue for the Ene Reaction between Maleimide and Allylbenzenes

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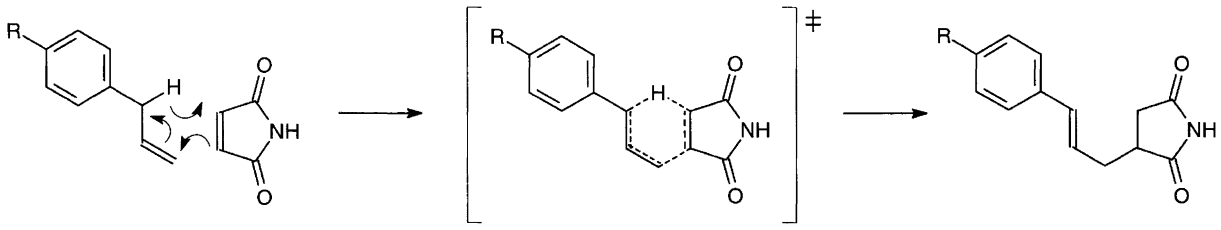
A bicyclic phenyldioxohexahydro-1*H*-isoindole-based hapten with a high similarity to the transition-state of the ene reaction between maleimide and allylbenzenes has been designed and synthesized.

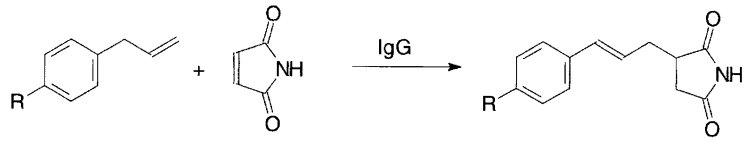
The thermal (or Lewis acid catalysed) ene reaction between an allylic hydrogen-possessing olefin and a compound containing a double (or triple) bond produces a new carbon–carbon bond with migration of the ene double bond and 1,5-hydrogen shift.[1] The concerted ene reaction proceeds through a cyclic, six-electron transition-state (Scheme 1). Thus, it resembles mechanistically the Diels–Alder reaction. As ene reactions are generally carried out at temperatures in excess of 150 °C, their synthetic use is limited to only very stable alkenes and enophiles. Our target ene reaction between various allylbenzenes **1** and maleimide **2** has a relatively high activation energy (Scheme 2; R = H, AcNH, MeO).[2] A catalytic antibody with its ability to lower the energy of activation of a chemical reaction may allow the ene reaction to be run under less extreme conditions. It is widely accepted that an energy barrier of approximately 84 kJ mol^{-1} (20 kcal mol^{-1}) is the maximum binding energy that an antibody is able to utilise in catalysing pericyclic reactions.[3] Therefore, we embarked on a calculation at AM1 level, modelling the six-membered pericyclic transition-state of this reaction[2] and the synthesis of a stable analogue of the transition-state for eliciting monoclonal antibodies. This short paper describes a convenient method for the preparation of a bicyclic transition-state analogue **10**.

Results and discussion

A retrosynthetic analysis of the target molecule **10** reveals an obvious Diels–Alder disconnection of the cyclohexene ring system. This disconnection gives the 2-phenyl-1,3-



Scheme 1. The transition-state of the ene reaction between maleimide and substituted allylbenzenes.



1 **2** **3**

Scheme 2.

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butadiene and maleimide synthons, which are readily available for starting materials. Commercially available 4-aminoacetophenone **4** was protected as a *tert*-butyl carbamate **5** (Scheme 3). The Grignard reaction of carbamate **5** with vinylmagnesium bromide in tetrahydrofuran gave the tertiary alcohol **6** in 53% yield. The one-pot mesylation and elimination procedure of **6** using methanesulfonyl chloride in triethylamine with 4-dimethylaminopyridine as a nucleophilic catalyst in dichloromethane afforded 2-phenyl-1,4-butadiene **7** in a surprisingly poor yield (4%). However, the elimination procedure of **6** using the one-pot treatment with triflic anhydride and 2,6-lutidine in dichloromethane gave diene **7** in 69% yield.[4]

A subsequent Diels–Alder reaction of 2-phenylbutadiene **7** with maleimide **2** in toluene at 50 °C furnished the cycloadduct **8** in a very good yield (87%). The *tert*-butoxycarbonyl protecting group of **8** was removed by standard treatment with trifluoroacetic acid in dichloromethane to give the amine **9** as its trifluoroacetate. Acylation of **9** with glutaric anhydride in the presence of triethylamine in dichloromethane afforded the hapten **10** in 74% yield.

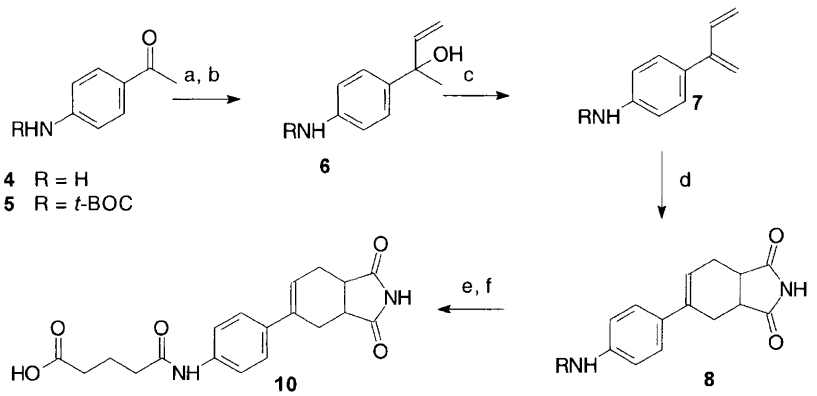
The hapten **10** was activated for the coupling with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-hydroxysulfosuccinimide (NHS).[5] The activated hapten was coupled to keyhole limpet hemocyanin (KLH) and the resultant immunoconjugate was used to immunise Balb/c mice for the production of monoclonal antibodies. The activated hapten was also coupled to bovine serum albumin (BSA) for screening purposes. Six monoclonal antibodies for the hapten **10** were shown by an enzyme-linked immunosorbent assay[6] (ELISA) to bind to **10** conjugated to bovine serum albumin. Taken together, we have shown that the bicyclic phenyldioxohexahydro-1*H*-isoindole hapten **10** coupled to KLH is able to induce an immune response and elicit monoclonal antibodies that recognise the cyclic, six-electron transition-state of an ene reaction. We are currently studying these monoclonal antibodies for their potential catalytic properties and the results of these studies will be reported in due course.

Experimental

General methods. Unless otherwise stated, reactions were carried out in oven-dried or flame-dried glassware under a positive atmosphere of argon. Reagents were transferred with plastic syringes and oven-dried or disposable needles. Commercial-grade reagents were used without further purification except as indicated below. Tetrahydrofuran was distilled from sodium–benzophenone ketyl. Dichloromethane, 1,4-dioxane, triethylamine, and toluene were distilled from calcium hydride. All chromatography solvents were obtained commercially and used without further purification. Methanesulfonyl chloride and 2,6-lutidine were purified by distillation under diminished pressure. Grignard reagents were titrated in tetrahydrofuran with 2-butanol using 1,10-phenanthroline as an indicator.[7] Yields are for unoptimised procedures and refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise noted.

^{1}H NMR spectra (600 MHz) were recorded on a Varian UNITY 600 instrument using $CDCl_3$ or ethanol-d_6 as solvents. Chemical shifts were reported as δ units (ppm) downfield from tetramethylsilane (TMS) using residual solvent signals as internal standards: δ 7.26 ($CDCl_3$): δ 3.55, 1.10 (ethanol-d_6). High and low resolution fast atom bombardment mass spectra were provided by the Mass Spectrometry Facility of the Technical Research Centre of Finland.

4-[(1,1-Dimethylethoxy)carbonylamino]acetophenone (**5**). A mixture of 4-aminoacetophenone **4** (5.00 g, 37.0 mmol), di-*tert*-butyl dicarbonate (8.88 g, 40.7 mmol) and 1,4-dioxane (70 ml) was stirred overnight at room temperature. The reaction mixture was evaporated *in vacuo* and chromatography of the residue on silica gel (EtOAc–*n*-hexanes 1:1) gave **5** as an off-



Scheme 3. (a) $(tBOC)_2O$ 1.1 equiv., 1,4-dioxane, r.t., overnight, 93%; (b) CH_2=CHMgBr in THF 2.1 equiv., THF, 0 °C, 30 min, 53%; (c) Tf_2O 1 equiv., 2,6-lutidine 4.6 equiv., CH_2Cl_2, −10 °C, 2 h, 69%; (d) maleimide **2** 1.2 equiv., PhMe, 50 °C, 4 h, 87%; (e) TFA (xs), CH_2Cl_2, r.t., 30 min, 63%; (f) glutaric anhydride 2 equiv., TEA 2 equiv., CH_2Cl_2, r.t., 16 h, 74%.

white solid (8.13 g, 93%). ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9 H), 2.56 (s, 3 H), 6.76 (s, 1 H), 7.45 (d, J=8.0 Hz, 2 H), 7.91 (d, J=8.0 Hz, 2 H). HRMS: m/z calcd. for $C_{13}H_{17}NO_3$ 235.1204, found 235.1203.

1-{4-[(1,1-Dimethylethoxy)carbonylamino]phenyl}-1-methyl-2-propen-1-ol (**6**). A 1.0 M solution of vinylmagnesium bromide in tetrahydrofuran (44.6 ml, 44.6 mmol) was added dropwise to a stirred solution of **5** (5.00 g, 21.3 mmol) in tetrahydrofuran (30 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The reaction was quenched by addition of 1 M HCl solution (50 ml) and stirring of the reaction mixture at room temperature for 10 min. The mixture was extracted with EtOAc (3 × 60 ml) and the combined organic layers were washed with a saturated aqueous solution of NH_4Cl (30 ml) and brine (30 ml), dried with anhydrous $MgSO_4$, filtered and evaporated *in vacuo*. The crude residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH 95:5) to afford **6** as an off-white solid (2.98 g, 53%). ^{1}H NMR ($CDCl_3$): δ 1.51 (s, 9 H), 1.63 (s, 3 H), 1.86 (br s, 1 H), 5.12 (d, J=11 Hz, 1 H), 5.27 (d, J=17 Hz, 1 H), 6.14 (dd, J=11 and 17 Hz, 1 H), 6.48 (s, 1 H), 7.12 (d, J=8.0 Hz, 2 H), 7.38 (d, J=8.0 Hz, 2 H). LRMS: m/z 263 and 286 [$M+Na^+$]. HRMS: m/z calcd. for $C_{15}H_{21}NO_3$ 263.1516, found 263.1508.

2-{4-[(1,1-Dimethylethoxy)carbonylamino]phenyl}-1,3-butadiene (**7**). To a CH_2Cl_2 (20 ml) solution of the methyl vinyl alcohol **6** (1.30 g, 4.94 mmol) was added 2,6-lutidine (2.64 ml, 2.43 g, 22.7 mmol) and trifluoromethanesulfonic anhydride (0.83 ml, 1.39 g, 4.94 mmol) at −10 °C and the mixture was stirred at the same temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (100 ml) and washed with brine (40 ml). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 ml) and the combined organic layers were dried with anhydrous $MgSO_4$, filtered, evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (*n*-hexanes–EtOAc 3:1) to afford **7** as a slightly yellowish solid (0.84 g, 69%): ^{1}H NMR ($CDCl_3$): δ 1.54 (s, 9 H), 5.17 (s, 1 H), 5.20 (d, J=11 Hz, 1 H), 5.21 (d, J=17 Hz, 1 H), 5.25 (s, 1 H), 6.48 (br s, 1 H), 6.60 (dd, J=11 and 17 Hz, 1 H), 7.26 (d, J=8.0 Hz, 2 H), 7.38 (d, J=8.0 Hz, 2 H). LRMS: m/z 245. HRMS: m/z calcd. for $C_{15}H_{19}NO_2$ 245.1411, found 245.1413.

5-{4-[(1,1-Dimethylethoxy)carbonylamino]phenyl}-1,3-dioxo-2,3,3a,4,7,7a-hexahydro-1H-isoindole (**8**). Diene **7** (138 mg, 0.56 mmol) was added to a solution of maleimide (65.2 mg, 0.67 mmol) in toluene (5 ml). The reaction mixture was stirred at 50 °C for 4 h and evaporated *in vacuo*. The residue was purified by silica gel chromatography (*n*-hexane–EtOAc 1:1) to yield **8** as an off-white solid (167 mg, 87%). ^{1}H NMR ($CDCl_3$): δ 1.51 (s, 9 H), 2.31–2.38 (obscure, 1 H), 2.50 (dd, J=7 and 16 Hz, 1 H), 2.78 (ddd, J=3, 7 and 16 Hz, 1 H), 3.09 (dd, J=3 and 16 Hz, 1 H), 3.19 (m, 1 H), 3.29 (m, 1 H), 6.11 (m, 1 H), 6.58 (br s, 1 H), 7.24–7.31 (overlap with residual signal of the solvent), 8.20 (br s, 1 H). LRMS: m/z 365 [$M+Na^+$]. HRMS: m/z calcd. for $C_{19}H_{22}N_2O_4$ 342.1574, found 342.1576.

5-(4-Aminophenyl)-1,3-dioxo-2,3,3a,4,7,7a-hexahydro-1H-isoindole trifluoroacetate (**9**). A mixture of the Diels–Alder adduct **8** (157 mg, 0.46 mmol) and trifluoroacetic acid (2.00 ml, 2.97 g, 26.0 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 30 min. The reaction mixture was evaporated *in vacuo* to give **9** as an off-white powder (102 mg, 63%) that gave a positive ninhydrin test. ^{1}H NMR ($CDCl_3$): δ 2.33 (m, 1 H), 2.48 (m, 1 H), 2.77 (ddd, J=3, 7 and 16 Hz, 1 H), 3.08 (dd, J=3 and 16 Hz, 1 H), 3.19 (ddd, J=3, 8 and 9 Hz, 1 H), 3.28 (ddd, J=3, 7 and 9 Hz, 1 H), 6.04 (m, 1 H), 6.63 (d, J=9 Hz, 2 H), 7.17 (d, J=9 Hz, 2 H), 7.83 (br s, 1 H). LRMS: m/z 243 [$M+H^+$].

5-[4-(1,3-Dioxo-2,3,3a,4,7,7a-hexahydro-1H-isoindol-5-yl)phenylamino]-5-oxopentanoic acid (**10**). A mixture of **9** (102 mg, 0.29 mmol), glutaric anhydride (67 mg, 0.59 mmol), triethylamine (82 µl, 60 mg, 0.59 mmol) and CH_2Cl_2 (5 ml) was stirred at room temperature for 16 h. The reaction mixture was evaporated *in vacuo*. The crude product was purified by silica gel chromatography (EtOAc–AcOH 100:1) and recrystallised from EtOH to give **10** as a white solid (76 mg, 74%), which gave a negative ninhydrin test. The product gave a single peak (7.0 min) on HPLC (Unisil Pack 250B type column, 6.0 × 250 mm, GL Sciences Inc., eluent 33% MeCN–H_2O, 0.6 ml min^{-1}; detected at 220 nm). ^{1}H NMR (ethanol-d_6): δ 1.96 (quintet, J=7 Hz, 2 H), 2.33 (m, 4 H), 2.42 (t, J=7 Hz, 2 H), 2.49 (dd, J=7 and 16 Hz, 1 H), 2.71 (ddd, J=2, 7 and 16 Hz, 1 H), 3.05 (dd, J=2 and 16 Hz, 1 H), 3.19 (ddd, J=2, 7 and 9 Hz, 1 H), 3.30 (ddd, J=2, 7 and 9 Hz, 1 H), 6.10 (m, 1 H), 7.26 (d, J=9 Hz, 2 H), 7.54 (d, J=9 Hz, 2 H). LRMS: m/z 356 [M^+]. HRMS: m/z calcd. for $C_{19}H_{20}N_2O_5$ 356.1367, found 356.1359.

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References

1. For some general reviews on the ene reaction, see: (a) Hoffmann, H. M. R. *Angew. Chem. 81* (1969) 597; (b) Oppolzer, W. *Angew. Chem., Int. Ed. Engl 23* (1984) 876; (c) Mikami, K. and Shimizu, M. *Chem. Rev. 92* (1992) 1021.
2. (a) Yliniemelä, A., Konschin, H., Pietilä, L.-O. and Teleman, O. *J. Mol. Struct. (Theochem) 334* (1995) 173; (b) Yliniemelä, A., Konschin, H., Neagu, C., Pajunen, A., Hase, T., Brunow, G. and Teleman, O. *J. Am. Chem. Soc. 117* (1995) 5120.

3. (a) Gouverneur, V. E., Houk, K. N., de Pascual-Teresa, B., Beno, B., Janda, K. D. and Lerner, R. A. *Science 262* (1993) 204; (b) Yli-Kauhaluoma, J. T., Ashley, J. A., Lo, C.-H., Tucker, L., Wolfe, M. M. and Janda, K. D. *J. Am. Chem. Soc. 117* (1995) 7041; (c) Heine, A., Stura, E. A., Yli-Kauhaluoma, J. T., Gao, C., Deng, Q., Beno, B. R., Houk, K. N., Janda, K. D. and Wilson, I. A. *Science 279* (1998) 1934.
4. Naruta, Y., Nagai, N., Arita, Y. and Maruyama, K. *J. Org. Chem. 52* (1987) 3956.
5. Staros, J. V. *Biochemistry 21* (1982) 3950.
6. Engvall, E. *J. Immunol. 109* (1972) 129.
7. Watson, S. C. and Eastham, J. F. *J. Organomet. Chem. 9* (1967) 165.

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