

Supplementary Material

Gelatin-based hydrogels through homobifunctional triazolinediones targeting tyrosine residues

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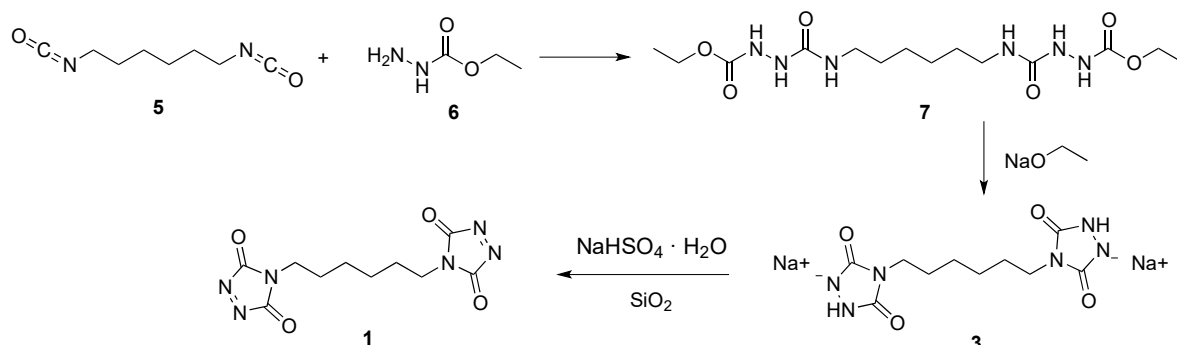
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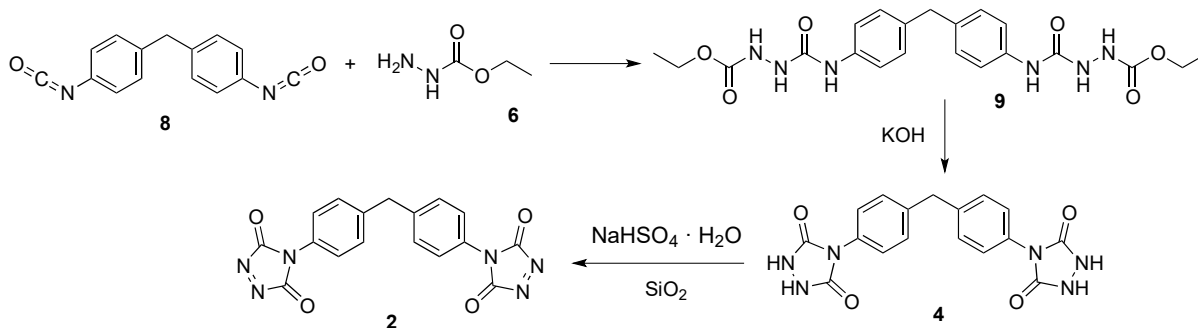
References

Synthesis of cross-linking agents

Synthesis of TAD 1



Synthesis of TAD 2



Scheme S1. Synthetic scheme for the synthesis of TAD 1 and 2.

The methodologies for the synthesis of TAD 1 and 2 were adapted from the procedures reported by Culbertson and McGrath [1]. The procedure by Mallakpour and co-worker was used for the oxidation of urazoles 3 and 4 [2].

Diethyl 4,13-dioxo-2,3,5,12,14,15-hexaazahexadecane-1,16-dioate (7). Ethylcarbazate **6** (2.08 g, 20 mmol) was dissolved in 100 mL of dry toluene, under nitrogen atmosphere. The solution was cooled to 0°C, then 1,6-diisocyanate hexane **5** (1.68g, 10 mmol), in 10 ml of dry toluene, was added over a period of 10 min. The mixture was rinsed to r.t. and stirred for 30 min, then refluxed for 3 h. After cooling to r.t., the product was recovered by filtration, washed with cold toluene, and dried at 80°C overnight (3.5 g, 94%). The physical and spectroscopic data corresponded to those reported in the literature [1].

Diethyl 2,2'-[methylenebis(4,1-phenyleneiminocarbonyl)]dihydrazinecarboxylate (9). Ethylcarbazate **6** (2.08 g, 20 mmol) was dissolved in 100 mL of dry toluene, under nitrogen atmosphere. The solution was cooled to 0°C, then 1,1'-methylenebis(4-isocyanatobenzene) **8** (2.5g, 10 mmol), in 10 ml of dry toluene, was added over a period of 10 min. The mixture was rinsed to r.t. and stirred for 30 min, then refluxed for 3 h. After cooling to r.t., the product was recovered by filtration, washed with cold toluene, and dried at 80°C overnight (4.4 g, 96%). The physical and spectroscopic data corresponded to those reported in the literature [1].

1,6-Hexanebisurazole di-sodium salt (3). **7** (1.89 g, 5 mmol) and NaOEt (0.85 g, 12.5 mmol) were added to EtOH (20 mL), under nitrogen atmosphere. The reaction mixture was then heated for 24h. After 1h a clear orange solution was formed and after few hours a yellowish solid start to precipitate. The residue was collected by filtration and dried overnight at 80°C under vacuum (10 torr) affording **3** (1.6 g, 98%). The physical and spectroscopic data corresponded to those reported in the literature [1].

4,4'-[methylenebis(4,1-phenylene)]bis(1,2,4-triazolidine-3,5-dione) (**4**). **9** (4 g, 8.7 mmol) in KOH aq (25 mL, 4 M) was heated at 100 °C for 5 h. The mixture was then cooled with an ice bath and acidified with concentrated HCl (37%) until pH ≈ 1. The residue was collected by filtration and dried overnight at 80°C under vacuum (10 torr) (2.75 g, 86%). The physical and spectroscopic data corresponded to those reported in the literature [1].

4,4'-hexane-1,6-diylbis(3H-1,2,4-triazole-3,5(4H)-dione) (**1**) and *4,4'*-[methylenebis(4,1-phenylene)]bis(3H-1,2,4-triazole-3,5(4H)-dione) (**2**). Urazole (1 mmol) was grinded into a mortar with NaHSO₄ · H₂O (8 equiv) and SiO₂ (400 mg, 70 - 200 μm). Water (200 μl) was added to the fine powder obtained, and the resulting mixture was stirred for 5 min. CH₂Cl₂ (25 mL) was then added and the suspension was cooled to 0 °C and kept in the dark for the whole reaction time. Every 30 min NaNO₂ (2 equiv) was added to the reaction mixture (8 eq. overall). The inorganic salts were removed by filtration and the solvent was removed under reduced pressure affording **1** and **2** in a pure form (210 mg, 75% for **1**; 217 mg, 60% for **2**). The physical and spectroscopic data corresponded to those reported in the literature [2].

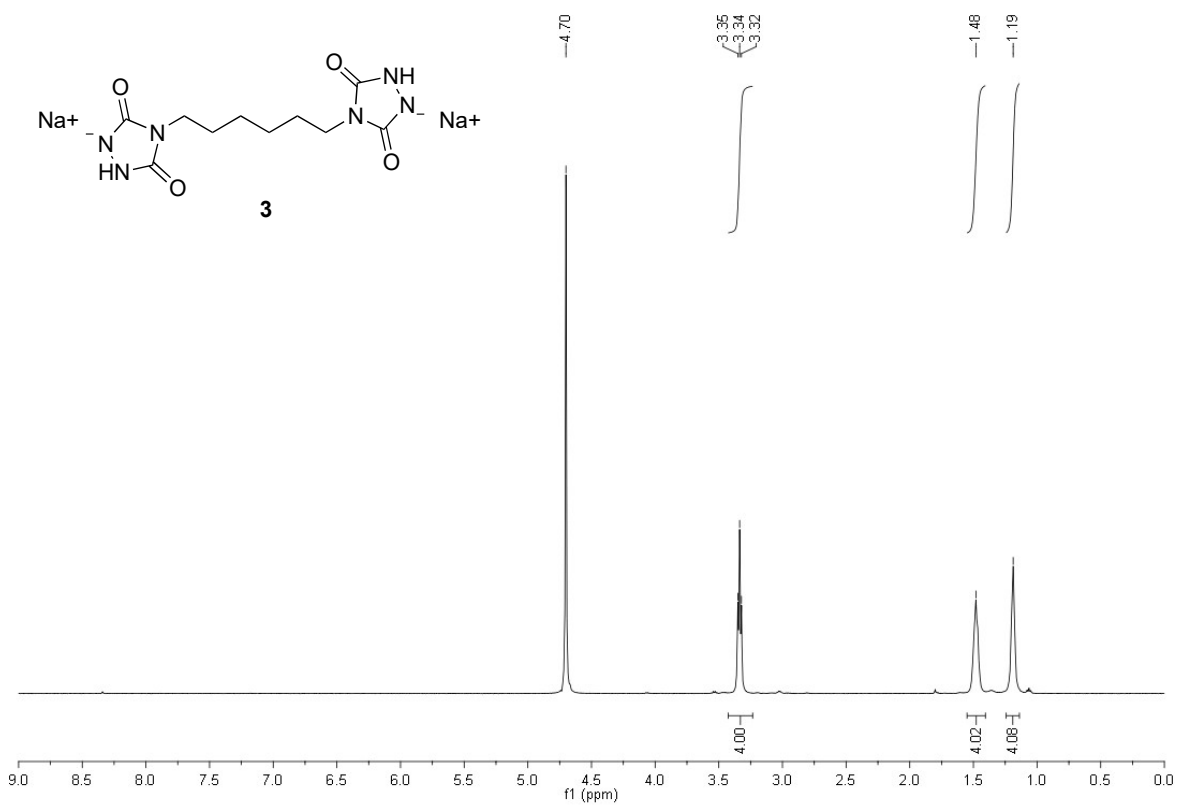


Figure S1. ¹H NMR of urazole 3.

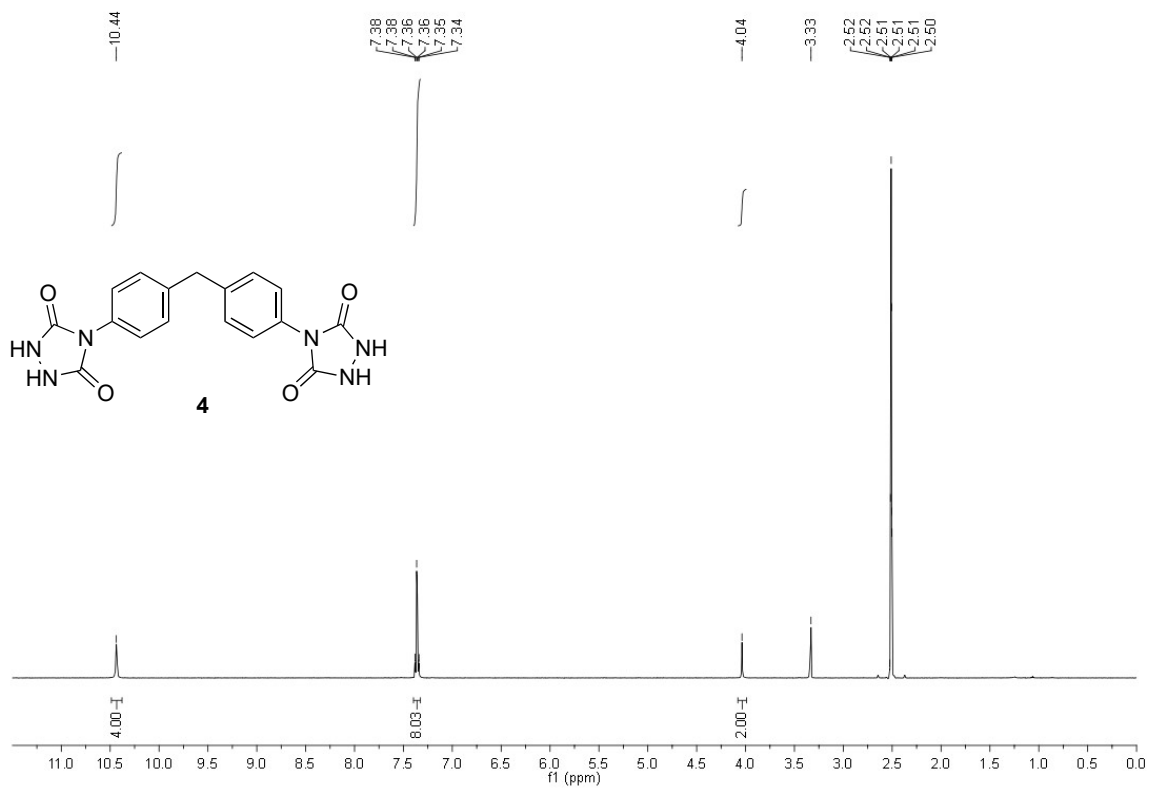


Figure S2. ¹H NMR of urazole 4.

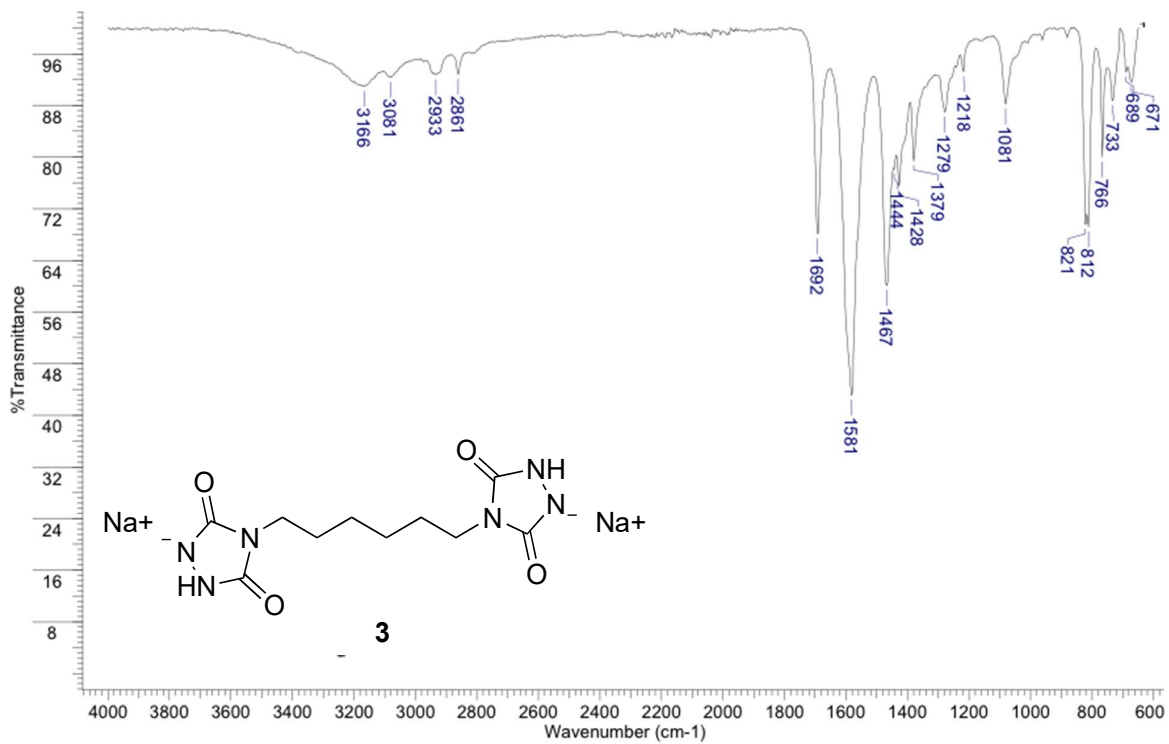


Figure S3. ATR-FTIR of urazole 3.

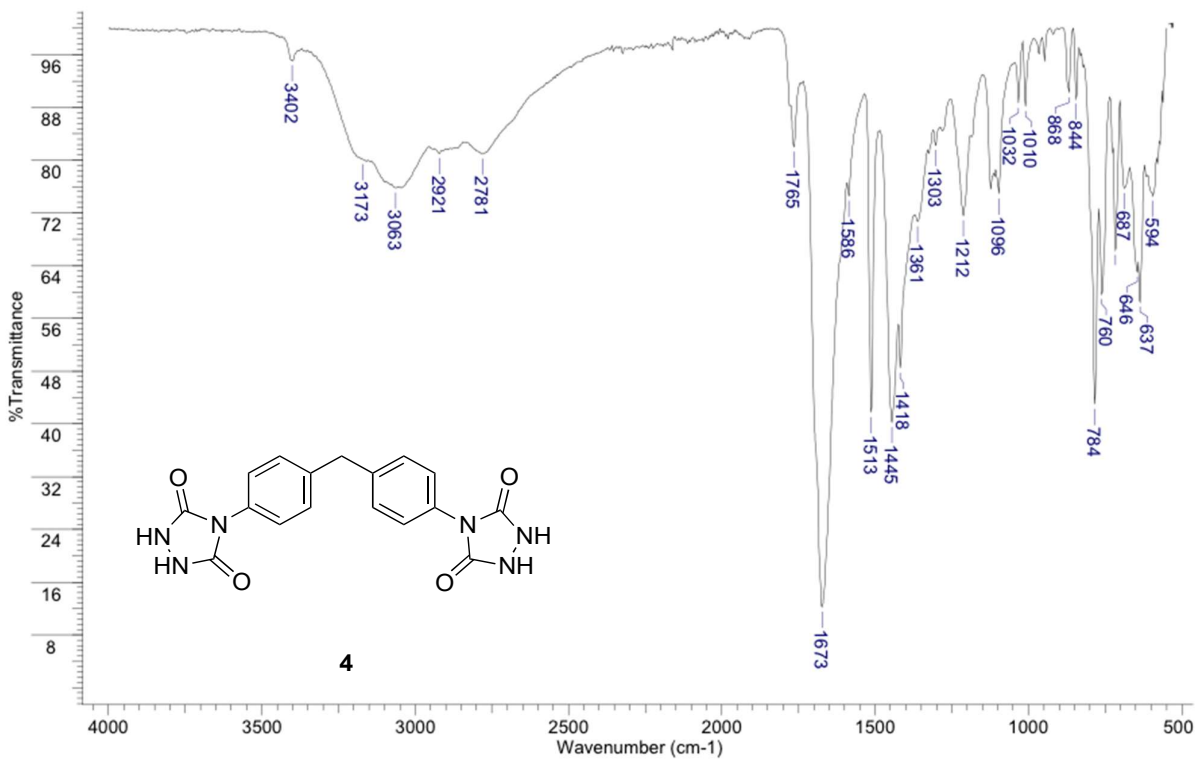


Figure S4. ATR-FTIR of urazole 4.

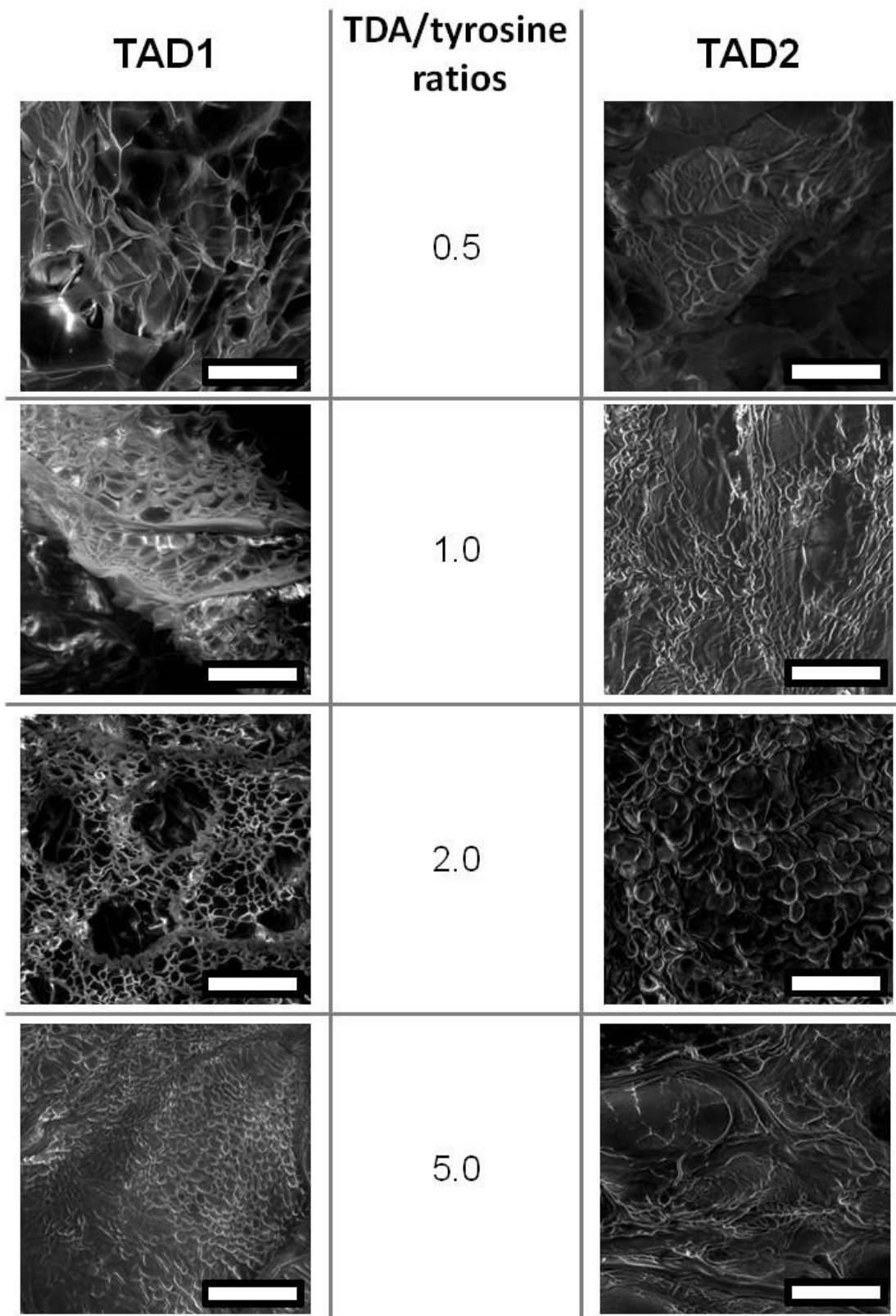


Figure S5. Representative SEM images for sample Gel_TAD1 e Gel_TAD2 at different TAD/tyrosine ratios. All the images were collected at the same magnification and scale bar is 50 nm.

References

- [1] Culbertson, B.M.; McGrath, J. E.; Polymer science and technology; Plenum Press New York; 1985; Volume 31, pp. 8-11, ISBN:13:978-1-4612-9254-8.
- [2] Zolfigol, M. A.; Mallakpour, S. E.; Madrakian, E.; Ghaemi, E.; Oxidation of urazoles to their corresponding triazolinediones under mild and heterogeneous conditions. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2000**, 39, 308-310, 2000:780195.