Bioorganic & Medicinal Chemistry 26 (2018) 4841–4849

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

The synthesis of novel chromogenic enzyme substrates for detection of bacterial glycosidases and their applications in diagnostic microbiology

Michael Burton[a], John D. Perry[b], Stephen P. Stanforth[c], Hayley J. Turner[a,*]

[a] Glycosynth Ltd, 14 Craven Court, Winwick Quay, Warrington, Cheshire WA2 8QU, UK
[b] Department of Microbiology, Freeman Hospital, Newcastle upon Tyne NE7 7DN, UK
[c] Department of Applied Sciences, Northumbria University, Newcastle upon Tyne NE1 8ST, UK

ARTICLE INFO

Keywords:
Enzyme substrates
Chromogenic substrates
Glycosidase
Pathogenic microorganisms
Bacterial detection

ABSTRACT

The preparation and evaluation of chromogenic substrates for detecting bacterial glycosidase enzymes is reported. These substrates are monoglycoside derivatives of the metal chelators catechol, 2,3-dihydroxynaphthalene (DHN) and 6,7-dibromo-2,3-dihydroxynaphthalene (6,7-dibromo-DHN). When hydrolysed by appropriate bacterial enzymes these substrates produced coloured chelates in the presence of ammonium iron (III) citrate, thus enabling bacterial detection. A β-D-riboside of DHN and a β-D-glucuronide derivative of 6,7-dibromo-DHN were particularly effective for the detection of *S. aureus* and *E. coli* respectively.

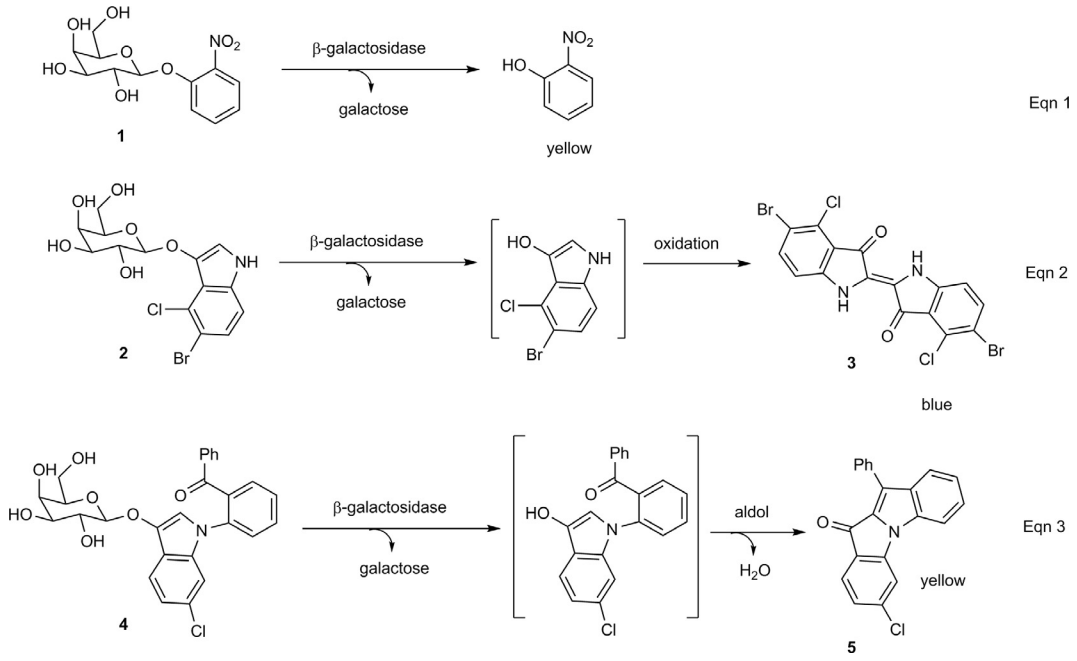
1. Introduction

Synthetic enzyme substrates are utilised extensively in diagnostic clinical microbiology for the purpose of detecting and identifying pathogenic microorganisms.[1–3] These substrates are designed to target microbiological species of interest (or groups of species) based upon their enzyme activity. An important sub-class of synthetic enzyme substrates are the chromogenic sugar-based enzyme substrates in which hydrolytic cleavage of the sugar moiety from the aglycone is mediated by an appropriate enzyme resulting in the liberation of a hydroxyaryl derivative, as shown by the representative examples in Scheme 1. The hydroxyaryl derivative can be coloured, thus allowing direct visualisation of the hydrolytic reaction as illustrated by the transformation of the colourless *ortho*-nitrophenyl β-D-galactopyranoside **1** into the yellow-coloured *ortho*-nitrophenol (ONP) (Eqn 1).[4] Alternatively, if the liberated hydroxyaryl derivative is colourless, a subsequent chemical reaction can be employed to produce a coloured product. Thus, the β-galactoside derivative of 5-bromo-4-chloro-3-hydroxyindole ('X-gal') **2** is hydrolysed to produce 5-bromo-4-chloro-3-hydroxyindole which then undergoes an oxidative dimerization in air producing the blue-coloured indigo derivative **3** (Eq. 2).[5] The indole-derived substrate, ALDOL™ 455 **4**,[6] similarly generates a reactive 3-hydroxyindole intermediate which participates in a subsequent non-oxidative intramolecular aldol condensation yielding the yellow chromophore **5** (Eq. 3). Sugar-based chromogenic substrates have also been designed around a glycosidated catechol moiety (Fig. 1). After enzymatic hydrolysis of the substrate, the resulting catechol aglycone undergoes chelation with metal ions that have been incorporated into the medium, therefore producing coloured metal-chelates. The hydrolysis of esculin **6** by a β-glucosidase enzyme yields D-glucose and esculetin (6,7-dihydroxycoumarin) which, in the presence of iron salts, produced a brown/black complex.[7] Cyclohexenoesculetin-β-D-glucoside **7**[8,9] (and also its β-D-galactoside derivative)[10] similarly generated a black complex in the presence of iron salts. Alizarin β-D-glucoside **8**[9] (and also its β-D-galactoside derivative)[11] yielded a purple-coloured chelate in the presence of iron salts and a pink-coloured chelate with aluminium salts. Hydrolysis of 3′,4′-dihydroxyflavone β-D-ribofuranoside[12] gave black colonies in the presence of iron and yellow colonies in the presence of aluminium. Other sugar-based substrates, which after enzymatic hydrolysis produce aglycones capable of chelation with metal ions, have also been prepared from non-catechol cores including glycosides of 8-hydroxyquinoline[13] and 3-hydroxyflavone.[9]

In this paper, we report the synthesis and microbiological evaluation of a series of novel chromogenic sugar-based enzyme substrates based upon catechol, 2,3-dihydroxynaphthalene and 6,7-dibromo-2,3-dihydroxynaphthalene **9** cores (Fig. 1).[14,15] 2,3-Dihydroxynaphthalene is inexpensive and available in large quantities (> 100 g) from several commercial suppliers thus making this an ideal starting material for the synthesis of enzyme substrates. Additionally, halogen atoms can be introduced into this ring-system remote from the hydroxyl-groups, i.e. at the 6,7-positions, whereas the introduction of halogen atoms into known substrates such as compounds **6** and **7** would only be possible

* Corresponding author.
E-mail address: steven.stanforth@northumbria.ac.uk (H.J. Turner).

https://doi.org/10.1016/j.bmc.2018.08.023
Received 18 May 2018; Received in revised form 1 August 2018; Accepted 16 August 2018
Available online 17 August 2018
0968-0896/ © 2018 Elsevier Ltd. All rights reserved.



Scheme 1. Hydrolysis of chromogenic enzyme substrates by β-galactosidase giving coloured products.

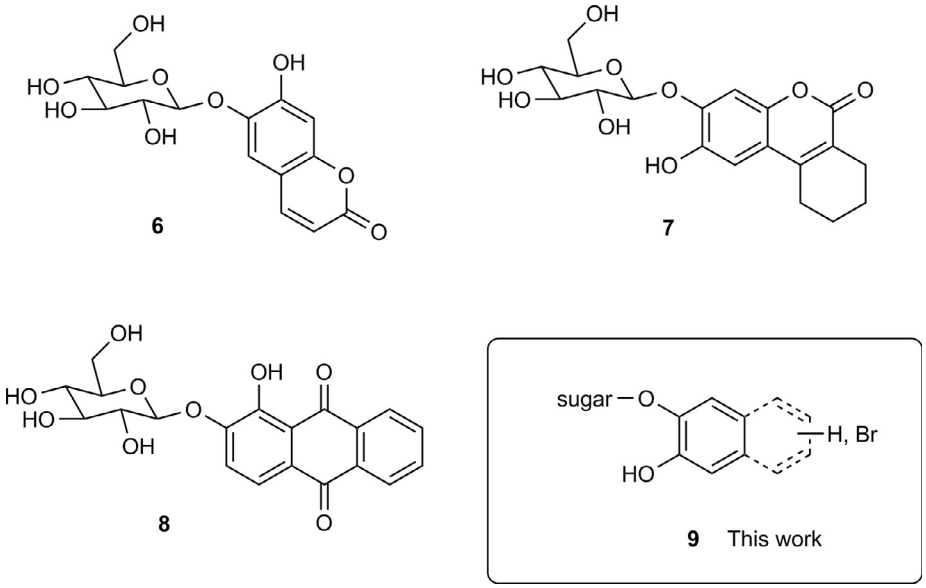


Fig. 1. Chromogenic enzyme substrates possessing a catechol moiety.

adjacent to the hydroxy-groups which may have a detrimental effect on glycosidase activity. We anticipated that the introduction of halogen atoms would be beneficial for reducing diffusion of chelates in solid (agar) media. We envisaged that catechol-derived substrates would have potential applications in liquid media (where the resulting metal chelates would require appreciable aqueous solubility) and that the increased size of the naphthalene-derived substrates **9** would potentially generate a more insoluble end-point better suited for use in solid (agar) media, where diffusion of the chelate must be localised within colonies of microorganisms. The sugar components of structures **9** have been chosen to target a broad range of enzymatic activities across a range of clinically important pathogenic microorganisms. The sugar moieties together with illustrative applications in diagnostic microbiology include: (i) β-D-glucopyranosides (for the detection of enterococci and *Listeria monocytogenes*), (ii) β-D-galactopyranosides (for the detection of coliforms), β-D-glucuronides (for the detection of *Escherichia coli*), *N*-acetylhexosaminides (for the detection of the pathogenic yeast, *Candida albicans*) and β-D-ribofuranosides (for the detection of *Staphylococcus aureus*, including MRSA). Catechol β-D-ribofuranoside[16] has previously shown efficacy for *S. aureus* detection in liquid media.[17]

2. Synthesis of substrates

Catechol 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **10**[18–21] was prepared from catechol in low yield using a Michael-type glycosidation procedure (Scheme 2). A Zemplén deprotection of compound **10** gave the required β-glucosidase substrate **11**. The proton-NMR spectral data of compounds **10** and **11** were consistent with those reported in the literature with large anomeric coupling constants confirming the β-configurations at the anomeric centres.[20,22] The direct reaction of glucose and catechol has been reported to give a 95:5 ratio of α:β anomers

Download English Version:

https://daneshyari.com/en/article/10154841

Download Persian Version:

https://daneshyari.com/article/10154841

Daneshyari.com