ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2013, **49**, 2936

Received 4th October 2012,
Accepted 25th February 2013

DOI: 10.1039/c3cc37265k

www.rsc.org/chemcomm

2,3-Dicyclohexylsuccinimide as a directing/protecting group for the regioselective glycosylation or alkylation of purines†

Ayan Pal, Kerry J. Salandria, Joseph W. Arico, Mark K. Schlegel* and Larry W. McLaughlin*

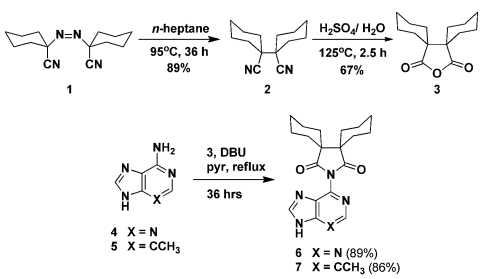
Here we describe the synthesis and application of a novel 2,3-dicyclohexylsuccinimide (Cy_2SI) protecting group towards regioselective purine glycosylation and alkylation reactions. This bulky protecting group promotes high regioselectivity during the glycosylation (as well as diastereoselectivity) or alkylation of purines using Hoffer's chlorosugar or *tert*-butyl bromoacetate, respectively. Cy_2SI offers the additional synthetic advantage that other base-labile protecting groups, such as toluoyl esters, can be selectively removed in its presence without affecting the imide.

Convergent approaches to synthesize nucleoside derivatives require a glycosylation event to couple the sugar and heterocycle components.[1] Typically, purine 2′-deoxynucleosides are prepared *via* the reaction of a 6-chloropurine heterocycle with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (Hoffer's chlorosugar)[2,3] employing the sodium salt method.[4] In this method, the anion of the purine base is generated at the tautomerically equivalent N9 and N7 positions, which commonly results in the presence of both N9 and N7 glycosylation products in the final product mixture. The use of 6-chloropurine as an acceptor results in a regioselectivity that favors the desired β-N9 product by an approximate factor of five.[1] This ratio, however, is reversed in the case of 3-substituted purines such as 6-chloro-3-deaza-3-methylpurine in which the formation of N7 products is favored.[5] An additional problem is that many purine acceptors typically generate notable quantities of the α-nucleoside products, further reducing yields and complicating purifications.[1]

Previous work by Robins and colleagues has described the use of purines bearing a 2-alkylimidazole[6,7] or triazole[7] in the 6-position of the purine heterocycle to control regio- and diastereoselectivity during glycosylation reactions. It was proposed that the 2-alkyl substituent covers the area over the N7 position of the purine heterocycle, sterically blocking access of the glycosyl donor to that site. One disadvantage of these reagents is that the purine substrates must be prepared from their corresponding oxopurines or chloropurines, thereby creating additional synthetic steps. Moreover, studies in our group have demonstrated that displacement of the imidazole/triazole derivative of 3-deazapurine nucleosides was difficult.[8]

We have previously described the use of 2,2,3,3-tetramethylsuccinimide (M_4SI) protected aminopurines during nucleoside synthesis.[8] This imide was quite effective in functioning both as a protecting group for the exocyclic C6-amino group, as well as a directing group to favor regioselective glycosylation at the N9 position of purines. Although we anticipated the corresponding phthalimide (NPhth) would function similarly, the crystal structure of adenyl-N6-tetramethylsuccinimide suggested that it is not the imide carbonyl that blocks access to the N7 of the purine substrate, but rather the methyl groups present in M_4SI.[8] The phthalimide, lacking such methyl groups, was much less effective at directing the products towards the desired N9 regioisomer, especially when using modified purine acceptors.

With this in mind, we wanted to determine whether increasing the steric bulk above the purine N7 would further optimize the directing ability of substituted succinimide derivatives. Although a variety of protecting groups could be envisioned, we chose the bicyclohexyl derivative **3** owing to the readily available starting material. We could easily convert 1,1-azobis(cyclohexanecarbonitrile) (**1**) to the corresponding bicyclohexyl-1,1′-dinitrile (**2**) (Scheme 1) in 89% yield under refluxing conditions in *n*-heptane.

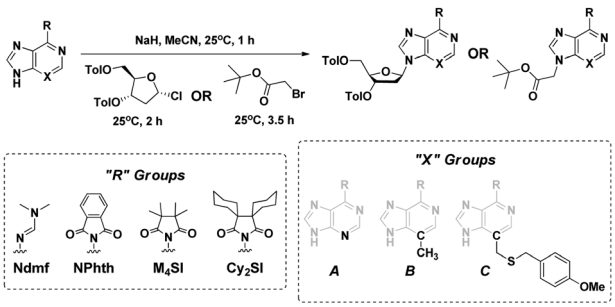


Scheme 1 Preparation of 2,3-dicyclohexylsuccinic anhydride and the subsequent protection of adenine or 3-deaza-3-methyladenine.

Boston College, Department of Chemistry, Merkert Chemistry Center, 2609 Beacon Street, Chestnut Hill, MA 02467, USA. E-mail: larry.mclaughlin@bc.edu, mark.schlegel@bc.edu; Fax: +001 617 552 2705; Tel: +001 617 552 3622

† Electronic supplementary information (ESI) available: Synthetic procedures, analytical data, oligonucleotide synthesis. CCDC 904677. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc37265k

Table 1 Regioselectivity and isolated yields for glycosylation and alkylation reactions



Purine substrate		Glycosylation reactions Entry	β-N9 : N7[b]	% Yield β9 (other)[c]	Alkylation reactions Entry	N9 : N7[b]	% Yield N9 (other)[c]
A	R = Ndmf[a]	1	0.8 : 1	18[e] (48)	14	0.7 : 1	30 (44)
	R = Cl	2[d]	4.7 : 1	57 (13)			
	R = NPhth	3[d]	>99 : 1	36 (0)			
	R = M_4SI	4[d]	>99 : 1	71 (6)			
	R = Cy_2SI	5	>99 : 1	94 (3)	15	15 : 1	78 (5)
B	R = Ndmf	6	0.7 : 1	6[e] (65)	16	0.5 : 1	0 (44)
	R = Cl	7[d]	0.6 : 1	39 (61)			
	R = NPhth	8[d]	0.9 : 1	15 (47)			
	R = M_4SI	9[d]	6.4 : 1	59 (27)			
	R = Cy_2SI	10	20 : 1	85 (12)	17	12 : 1	75 (6)
C	R = Ndmf	11	0.7 : 1	15[e] (35)	18	0.8 : 1	0 (52)
	R = M_4SI	12	7 : 1	57 (21)			
	R = Cy_2SI	13	16 : 1	82 (10)	19	10 : 1	71 (8)

[a] For synthesis, see ref. 10. [b] Determined using ^{1}H NMR integration on the crude products. [c] Isolated yields. [d] See ref. 8. [e] Determined using ^{1}H NMR integration on an isolated mixture of β-N9 and β-N7 products.

The dinitrile **2** was then subjected to strongly acidic conditions[9] to form the corresponding cyclic anhydride **3** in 67% yield.

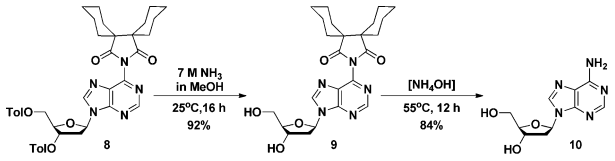
Introduction of this Cy_2SI protecting group to a series of 6-amino purines was accomplished in a single step by simply refluxing the purine, for example **4**, cyclic anhydride **3**, and DBU in pyridine to afford compound **6** in 89% yield. The crystal structure of compound **7** illustrates that the two cyclohexyl groups position themselves directly above the N7 of the purine base; specifically a methylene group from each of the cyclohexyl moieties hinders access to the purine N7 (see ESI†). We envisioned that this would make the use of Cy_2SI more advantageous towards regioselective purine reactions than M_4SI since the cyclohexyl groups flank both sides at the top of the purine nucleobase rather than just one in the case of M_4SI.[8]

To determine how effective Cy_2SI was in promoting regioselective glycosylations using Hoffer's chlorosugar, a series of reactions was performed in acetonitrile using the sodium salt method (Table 1). Glycosylation of adenine protected as the *N*-dimethylformamidine[10] (Ndmf) resulted in a complex mixture of β-N9 : β-N7 isomers in a ratio of 0.8 : 1, as well as a significant amount of the unwanted α isomers (Entry 1). Glycosylation using 6-chloropurine (which can be converted to adenine) resulted in a slightly higher β-N9 : β-N7 ratio of 4.7 : 1, but still contained a fair amount of α isomeric products (Entry 2). When the exocyclic amine of adenine was protected using phthalimide (NPhth), M_4SI, or Cy_2SI cyclo-imide protecting groups, the desired β-N9 products were formed in a greater than 99 : 1 ratio with respect to the β-N7 products (Entries 3–5). However, the isolated yields increased as the amount of steric bulk was increased in the cyclo-imide protecting group; the use of Cy_2SI significantly improved the isolated yield of the desired β-N9 product. Clearly, one big advantage of using Cy_2SI protection during glycosylation was that the resulting product mixture was more homogeneous, and its purification therefore much simpler, than a product mixture which potentially contained a complex blend of four compounds (N9/N7 regioisomers, α/β anomers).

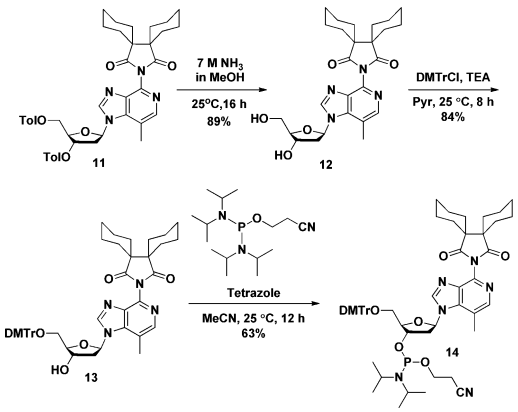
Encouraged by those results, we wanted to investigate the influence of Cy_2SI protection on glycosylation reactions of purine-like substrates in which the N3 in the purine heterocycle is replaced with a C–CH_3 (substrate set "B"). It had been previously shown that the introduction of a methyl group at the C3 position had a dramatic effect on both product ratios and isolated yields.[8] While the β-N9 : β-N7 ratio was found to favor the undesired β-N7 regioisomer for the Ndmf, 6-chloro, and NPhth protected derivatives (Entries 6–8), the use of the M_4SI-protected derivative (Entry 9) provided a regioselectivity (6.4 : 1) which favored the desired β-N9 regioisomer. Interestingly, the Cy_2SI-protected derivative performed *substantially* better with a regioselectivity of 20 : 1 during this reaction (Entry 10). Moreover, the isolated yield of the desired product was significantly higher with the Cy_2SI derivative when compared to the use of M_4SI (85% *vs.* 59%).

Acceptors containing more bulk attached to the C3 position (formerly N3 of adenine) of the substrate would appear to offer a more significant challenge under these glycosylation conditions by also sterically blocking the N9 position of the heterocycle (substrate set "C"). However, this group did not appear to have much of an overall effect. While the reactions with both M_4SI and Cy_2SI protection (Entries 12–13) showed a similar trend to that of the 3-deaza-3-methyladenine series (Substrate set "B," Entries 9–10), those using Cy_2SI protection showed a major improvement in both regioselectivity (16 : 1 *vs.* 7 : 1) and isolated yield (82% *vs.* 57%). Furthermore, it was also noteworthy that the purine heterocycles protected using Cy_2SI produced significantly lower amounts of the undesired α-isomers across all three substrate sets during these glycosylation reactions.

To expand the utility of Cy_2SI as a protecting/directing group, we also chose to study the alkylation reactions of this same set of purine substrates (Table 1). Studies have shown that alkylation reactions of purines also suffer from the production of N9 : N7 regioisomeric mixtures.[10,11] The ability to perform regioselective alkylation of purines would be advantageous for the production of N9 regioisomers in research fields that require them, such as peptide nucleic acids (PNA).[12] A simple alkylation of adenine, protected using Ndmf, with *tert*-butyl bromoacetate resulted in a 0.7 : 1 ratio of the N9 : N7 products (Entry 14). The use of Cy_2SI as a protecting group resulted in a dramatic reversal of this ratio, with the desired N9 regioisomer being produced in a 15-fold excess and isolated in 78% yield (Entry 15). Alkylation reactions of purine-like substrates with modifications at the 3-position of the heterocycle also followed the same trend observed in the corresponding glycosylation reactions; that is, Cy_2SI protection resulted in a 12 : 1 and 10 : 1 N9 : N7 ratio for substrates B and C, respectively (Entries 17 and 19). This was in stark contrast to the corresponding reactions using Ndmf protection in which the N7 regioisomer was formed in excess (Entries 16 and 18). It should



Scheme 2 Selective removal of toluoyl and Cy_2SI protecting groups.



Scheme 3 Synthesis of phosphoramidite **14**.

also be noted that although both the N9 and N7 regioisomers were detected in the crude ^{1}H NMR spectra for the corresponding heterocycles containing Ndmf protection (Entries 16 and 18), only the N7 product could be isolated *via* column chromatography.

The hydrolytic stability of the Cy_2SI protecting group also presented a synthetic advantage over other protecting groups. After glycosylation, the toluoyl esters of the newly formed nucleoside, for example **8**, could be selectively removed using 7 M ammonia in methanol at ambient temperature to produce compound **9** in 92% yield without affecting the Cy_2SI group (Scheme 2). Further reaction of compound **9** in concentrated aqueous ammonium hydroxide at 55 °C completely removed the imide protecting group and produced the free nucleoside **10** in 84% yield.

Encouraged by those results, we decided to test the feasibility of using Cy_2SI as a protecting group during DNA oligonucleotide synthesis. The synthesis of phosphoramidite **14** proceeded using well established procedures starting from compound **11** (Scheme 3). After removal of the toluoyl esters in **11** using 7 M NH_3 in MeOH, the free 5′-OH in **12** was selectively protected using 4,4′-dimethoxytrityl chloride in pyridine to afford compound **13** in 84% yield. Compound **13** was then transformed into phosphoramidite **14** using 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite and tetrazole in DCM in 63% yield. With this phosphoramidite in hand, we were able to successfully synthesize two 15-mer oligonucleotides which incorporated one or two modified nucleotides containing the Cy_2SI protecting group. However, after cleavage/deprotection and purification over a C_{18} Sep-Pak column[13] we observed exclusively the oligonucleotide product which still contained full Cy_2SI protection in both oligos (see ESI†). Further attempts at the removal of the Cy_2SI protecting group from the purified oligos with [NH_4OH], 7 M NH_3 in MeOH, or AMA (1 : 1 mixture of [NH_4OH] and 40% aq. methylamine) at 55 °C was unfortunately met with little success.

In summary, we have developed a new directing/protecting group that provided an effective means to access 6-aminopurine-2′-deoxynucleosides with a high regioselectivity for the desired β-N9 product over the β-N7 product. This Cy_2SI protecting group also provided this advantage to alkylation reactions in which the N9 product was formed in a significant excess over the undesired N7 product. Structural analysis using X-ray crystallography confirmed that portions of both cyclohexyl groups are positioned to sterically block access to the N7 nitrogen, thereby promoting reaction at the N9 position instead. This advantage became especially apparent in the synthesis of valuable C3 substituted purines in which the desired products could be easily isolated in high yields.[14] Since it has been previously shown that running purine glycosylation reactions in less polar solvents can reduce the number of α-nucleosides produced during the reaction,[15] further development of directing/protecting groups is ongoing in our laboratory.

We gratefully acknowledge funding from the NSF (MCB 0958515) for this research. We would also like to thank Dr Han Yueh and Ms Alena Carlson for their generous donation of synthetic intermediates.

Notes and references

1 L. J. Wilson, M. W. Hager, Y. A. El-Katan and D. C. Liotta, *Synthesis*, 1995, 1465–1479.
2 M. Hoffer, *Chem. Ber.*, 1960, **93**, 2777–2781.
3 V. Rolland, M. Kotera and J. Lhomme, *Synth. Commun.*, 1997, **27**, 3505–3511.
4 Z. Kazimierczuk, H. B. Cottam, G. R. Revankar and R. K. Robins, *J. Am. Chem. Soc.*, 1984, **106**, 6379–6382.
5 R. J. Irani and J. Santalucia, *Nucleosides Nucleotides Nucleic Acids*, 2002, **21**, 737–751.
6 M. Zhong, I. Nowak, J. F. Cannon and M. J. Robins, *J. Org. Chem.*, 2006, **71**, 4216–4221.
7 M. Zhong, I. Nowak and M. J. Robins, *J. Org. Chem.*, 2006, **71**, 7773–7779.
8 J. W. Arico, A. K. Calhoun, K. J. Salandria and L. W. McLaughlin, *Org. Lett.*, 2010, **12**, 120–122.
9 C. G. Overberg, H. Biletch, P. T. Huang and H. M. Blatter, *J. Org. Chem.*, 1955, **20**, 1717–1720.
10 K. Okumura, T. Oine, Y. Yamada, M. Tomie, T. Adachi, T. Nagura, M. Kawazu, T. Mizoguchi and I. Inoue, *J. Org. Chem.*, 1971, **36**, 1573–1579.
11 (*a*) T. Fujii and T. Itaya, *Heterocycles*, 1998, **48**, 1673–1724; (*b*) E. Enkvist, G. Raidaru, A. Uri, R. Patel, C. Redick, J. L. Boyer, J. Subbi and I. Tammiste, *Nucleosides Nucleotides Nucleic Acids*, 2006, **25**, 141–157; (*c*) Q. Zhang, G. Cheng, Y.-Z. Huang, G.-R. Qu, H.-Y. Niu and H.-M. Guo, *Tetrahedron*, 2012, **68**, 7822–7826.
12 (*a*) P. E. Nielsen, *Acc. Chem. Res.*, 1999, **32**, 624–630; (*b*) P. E. Nielsen, *Chem. Biodiversity*, 2010, **7**, 786–804; (*c*) E. Uhlmann, A. Peyman, G. Breipohl and D. W. Will, *Angew. Chem., Int. Ed.*, 1998, **37**, 2796–2823.
13 (*a*) M. K. Schlegel and E. Meggers, *J. Org. Chem.*, 2009, **74**, 4615–4618; (*b*) M. K. Schlegel, J. Hütter, M. Eriksson, B. Lepenies and P. H. Seeberger, *ChemBioChem*, 2011, **12**, 2791–2800.
14 K. J. Salandria, J. W. Arico, A. K. Calhoun and L. W. McLaughlin, *J. Am. Chem. Soc.*, 2011, **133**, 1766–1768.
15 (*a*) G. Ritzmann, R. S. Klein, D. H. Hollenberg and J. J. Fox, *Carbohydr. Res.*, 1975, **39**, 227–236; (*b*) H. G. Howell, P. R. Brodfuehrer, S. P. Brundlage, D. A. Benigni and C. Sapino, *J. Org. Chem.*, 1988, **53**, 85–88.

