Microwave-Assisted Selective 5′-*O*-Trityl Protection of Inosine Derivatives

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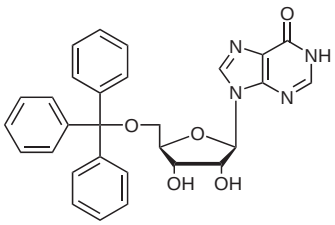
Received 12 March 2007

Abstract: The efficient microwave-assisted synthesis of 5′-*O*-trityl inosine derivatives is described. The reported protocol allows the protection of inosine derivatives in significantly higher yields and shorter reaction times than the standard thermal conditions. This procedure is particularly suitable for modified inosines where the 5′-OH is sterically hindered (i.e. 8-bromoinosine).

Key words: protecting groups, microwave synthesis, nucleosides, tritylether, medicinal chemistry



KIN 59 (**1**)

Figure 1 5′-*O*-Tritylinosine (KIN59) structure

The selective protection of the 5′-OH functionality in ribonucleosides by reaction with trityl chloride (TrCl) is one of the key steps in nucleoside chemistry, allowing manipulations at the 2′- and 3′-OH, as well as at the heterocyclic base moiety. As a protecting strategy, it is mandatory that the selectivity and the conversion ratios are in a range from good to excellent. However, for the natural nucleoside inosine, this protecting strategy traditionally affords poor yields of the desired 5′-*O*-tritylinosine. The first reports on the synthesis of 5′-*O*-tritylinosine made use of standard tritylation procedures, which involved reacting inosine with trityl chloride in a solvent mixture of pyridine–DMF at 40 °C or under reflux. Under these conditions, the yields ranged from 15%[1] to 27%.[2] Later reports indicated that the low yields in this transformation are probably due to the unusual solubility characteristics of inosine.[3,4]

Our interest on 5′-*O*-tritylinosine is based on our discovery that this compound, coded as KIN59 (Figure 1), is the first allosteric inhibitor of the angiogenic enzyme thymidine phosphorylase (TPase), with a remarkable antiangiogenic activity in the chorioallantoic membrane (CAM) assay.[5]

We have demonstrated that the antiangiogenic activity of KIN59 requires the intact trityl-containing nucleoside.[5] Therefore, it was mandatory to improve the yield of the 5′-O-tritylation reaction of inosine.

Our recently reported optimized synthesis of 5′-*O*-tritylinosine involved treatment of inosine with TrCl (1.7 equiv) in pyridine in the presence of DMAP (0.04 equiv), at 80 °C for 15 hours to afford the desired 5′-O-tritylated derivative in 41% yield.[5] When the reaction between inosine and trityl chloride was performed in acetonitrile in the presence of Et_3N, the major compounds were 2′,5′-di-*O*-tritylinosine[6] (21%) and 3′,5′-di-*O*-tritylinosine[6] (18%). Alternatively, when diisopropylamine was employed as the base in DMF, at 40 °C,[7] the major product was the unexpected 1-*N*-tritylinosine (30% yield).[8] In almost all these transformations, the starting inosine was the most abundant compound.

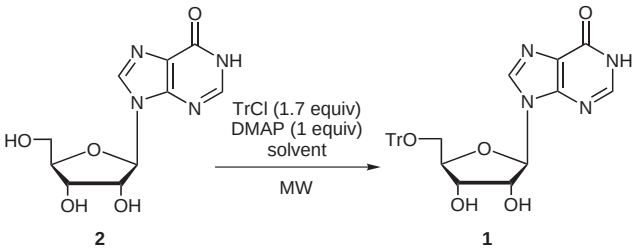
We therefore considered the use of microwave irradiation as a useful tool to enhance the selective 5′-O-trityl protection of inosine. Interestingly, despite the considerable interest in microwave-assisted organic synthesis (MAOS) for a variety of different processes,[9] we are aware of only one – relatively unsuccessful – previous attempt on the introduction of a trityl moiety at the 5′-OH of a nucleoside using microwave heating.[10]

As a starting point for the synthesis of 5′-*O*-tritylinosine using microwave irradiation, several of the most commonly used solvents for MAOS were screened, including DMF, MeCN, or DMSO. Different reaction times and bases were evaluated. The most relevant results are shown in Table 1.

Treatment of inosine with TrCl (1.7 equiv) in the presence of DMAP (1 equiv) in DMF at 80 °C for 60 minutes afforded 5′-*O*-tritylinosine in 64% yield, while longer reaction times did not result in a substantial improvement. Raising the temperature up to 100 °C or increasing the amount of DMAP to 2 equivalents, produced significant amounts of decomposition products. When MeCN was used as solvent, the yield of 5′-*O*-tritylinosine was only 40%. Changing the base from DMAP to imidazole in DMF also afforded lower yields. Interestingly, the reaction in DMSO produced mainly decomposition products.

SYNLETT 2007, No. 11, pp 1733–1735
Advanced online publication: 25.06.2007
DOI: 10.1055/s-2007-982532; Art ID: D07307ST

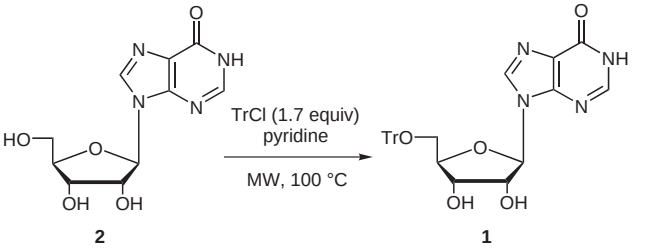
Table 1 Microwave-Assisted Tritylation of Inosine – Solvent Screening



Entry	Solvent	Temp (°C)	Time (min)	Yield (%)[a]
1	DMF	80	30	37
2	DMF	80	60	64
3	DMF	100	60	Dec.[b]
4	MeCN	80	60	40
5	DMSO	80	60	Dec.[b]
6[c]	Pyridine	80	60	50
7[c]	Pyridine	100	60	76

[a] Isolated yields of pure product after column chromatography.
[b] Only decomposition products.
[c] DMAP (0.04 equiv).

Table 2 Microwave-Assisted Tritylation of Inosine – Catalyst Effects



Entry	DMAP (equiv)	Time (min)	Yield (%)[a]
1	0.1	30	62
2	0.1	60	71
3	0.5	30	70
4	0.5	60	71
5	0.5	7	64
6	0.5	15	76

[a] Isolated yields of pure product after column chromatography.

To compare the thermal with the microwave conditions, we decided to use anhydrous pyridine as the solvent. The use of the best thermal conditions (1.7 equiv TrCl, 0.04 equiv DMAP, 80 °C, 15 h) under microwave irradiation afforded 5′-*O*-tritylinosine in 50% yield within 60 minutes (Table 1). When the same reaction was performed at 100 °C, the yield was improved up to 76%. Higher temperatures afforded undesired byproducts. We therefore selected pyridine as the solvent of choice for all subsequent studies.

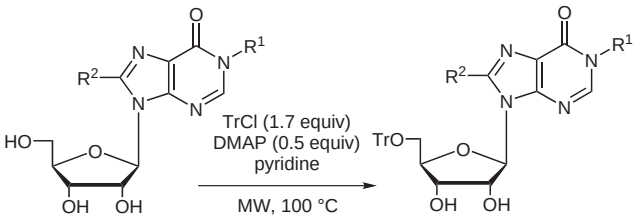
Substantial efforts were then made to optimize the amount of catalyst and the reaction time. The results are summarized in Table 2.

When the amount of DMAP was increased to 0.1 equivalents the yield was already 62% after 30 minutes (Table 2, entry 1). A longer reaction time (60 min, Table 2, entry 2) afforded a slightly better yield (71%). Interestingly, by increasing the amount of the DMAP catalyst from 0.1 equivalents to 0.5 equivalents a similar yield was obtained within 30 minutes (entry 3). Thus we examined shorter reaction times and the results indicated that after just 7 minutes of microwave irradiation, the yield of 5′-*O*-tritylinosine was already 64%, while 15 minutes reaction time led to a 76% isolated yield of 5′-*O*-tritylinosine. This yield could not be improved by further optimization, the major byproducts being di-*O*-trityl inosine derivatives (see above).

Next we focused on other inosine derivatives where the 5′-*O*-trityl protection is also problematic. For example, it has been reported that the introduction of a trityl moiety at the 5′-position of N^1-substituted inosines only provides moderate yields (from 34% to 43%) under standard conditions.[11] There is also a patent describing the preparation of N^1-benzyl-5′-*O*-tritylinosine in high yield, but requiring three days of reaction and 3.6 equivalents of trityl chloride, adding 1.2 equivalents every day.[12] We therefore decided to also perform the 5′-*O*-trityl protection reaction of N^1-substituted inosines under microwave conditions. The required N^1-substituted inosines were synthesized by reaction of inosine (200 mg, 0.74 mmol) in DMA (4 mL) with the corresponding alkyl or benzyl halide (0.92 mmol) in the presence of DBU (110 μL, 0.73 mmol) at room temperature overnight. In this way, N^1-allylinosine (**3**)[11] (90% yield) and N^1-benzylinosine (**4**)[13] (93% yield) were obtained. Treatment of **3** in pyridine with TrCl (1.7 equiv) in the presence of DMAP (0.5 equiv) at 100 °C under microwave conditions for 15 minutes afforded 1-allyl-5′-*O*-tritylinosine (**6**)[8] in 52% yield (Table 3, entry 1). By increasing the reaction time to 30 minutes, the yield was improved to 69% (Table 3, entry 2). Similarly, reaction of **4** with trityl chloride under analogous conditions afforded a 49% yield of 1-benzyl-5′-*O*-tritylinosine (**7**)[8] after 15 minutes, that was increased to 68% when the reaction was performed for 30 minutes (Table 3). When we compare the here described microwave-assisted protocol to our previously reported procedure involving thermal conditions,[8] the global yield starting from inosine is increased from 35% to 68% for the N^1-allyl derivative **6**, and from 27% to 71% for the N^1-benzyl derivative **7**.

Another interesting compound where the 5′-*O*-trityl protection has been problematic is 8-bromoinosine (**5**). The presence of the bromine at position 8 of the purine ring restricts the nucleoside conformation to the *syn*-form[14] where the nucleobase is positioned over the ribose

Table 3 Microwave-Assisted Tritylation of N^1-Substituted Inosines



3 R^1 = Allyl R^2 = H
4 R^1 = Bn R^2 = H
5 R^1 = H R^2 = Br

6 R^1 = Allyl R^2 = H
7 R^1 = Bn R^2 = H
8 R^1 = H R^2 = Br

Entry	Substrate	Time (min)	Product	Yield (%)[a]
1	**3**	15	**6**	52
2	**3**	30	**6**	69
3	**4**	15	**7**	49
4	**4**	30	**7**	68
5	**5**	15	**8**	40
6	**5**	30	**8**	71

[a] Isolated yields of pure product after column chromatography.

sugar and, as a consequence, the 5′-OH of the ribose is sterically hindered. Indeed, when the reaction between 8-bromoinosine[15] and trityl chloride was performed under standard thermal conditions (TrCl, pyridine, DMAP, 100 °C) a very poor 14% yield of 5′-*O*-trityl derivative was isolated. However, when the optimized microwave conditions were applied, the desired 8-bromo-5′-*O*-trityl-inosine (**8**)[16] was isolated in 40% yield after 15 minutes. If the reaction time was increased to 30 minutes, the yield was significantly improved up to 71%.

In conclusion, we have developed an efficient protocol for the selective 5′-*O*-trityl protection of inosine and structurally related analogues, using single mode microwave irradiation under controlled conditions. Our protocol allows higher conversions and considerable shorter reaction times compared to the previously published thermal conditions. The results contained in this letter will therefore be useful to other researchers in the field of inosine chemistry and nucleoside protection in general. In addition, the reported high-speed O-tritylation can be useful for other protective strategies involving the selective protection of primary versus secondary hydroxyl groups.

Typical Procedure for the Microwave-Assisted 5′-*O*-Trityl Protection of Inosine and Inosine Derivatives

In a 0.5–2.5 mL Pyrex microwave process vial, the appropriate inosine derivative (0.15 mmol), trityl chloride (0.25 mmol), DMAP (0.07 mmol), and anhyd pyridine (1.0 mL) were placed. The reaction vessel was sealed, stirred, and subsequently irradiated for 15 min at 100 °C in a single-mode microwave reactor (Emrys™ Synthesizer, Biotage AB). After cooling to ambient temperature, the mixture was loaded onto a silica gel column and purified by flash chromatography eluting with mixtures CH_2Cl_2–MeOH (100:0 to 100:10).

Acknowledgment

E.C. acknowledges the Consejería de Educación de la Comunidad de Madrid and the Fondo Social Europeo (F.S.E.) for a predoctoral fellowship. This work has been supported by grants from the Spanish MEC (SAF2006-12713-C02) and from the Christian Doppler Research Society (CDG).

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(13) Spectroscopic data for N^1-benzylinosine (**4**): ^{1}H NMR (360 MHz, DMSO-d_6): δ = 3.60 (m, 2 H, H-5′), 3.93 (m, 1 H, H-4′), 4.11 (m, 1 H, H-3′), 4.48 (m, 1 H, H-2′), 5.05 (t, *J* = 5.7 Hz, 1 H, OH), 5.21–5.23 (m, 3 H, CH_2, OH), 5.49 (m, 1 H, OH), 5.85 (d, *J* = 5.8 Hz, 1 H, H-1′), 7.24–7.36 (m, 5 H, Ph), 8.36, 8.62 (s, 2 H, H-2, H-8). MS (ES, positive mode): *m/z* = 358 $[M + 1]^+$.
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(16) Spectroscopic data for 8-bromo-5′-*O*-tritylinosine (**8**): white solid, mp 175–178 °C (MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.16 (m, 2 H, H-5′), 4.05 (m, 1 H, H-4′), 4.42 (m, 1 H, H-3′), 5.06 (m, 1 H, H-2′), 5.22 (d, *J* = 4.8 Hz, 1 H, OH), 5.56 (d, *J* = 5.7 Hz, 1 H, OH), 5.83 (d, *J* = 4.2 Hz, 1 H, H-1′), 7.19–7.30 (m, 15 H, Ph), 7.88 (s, 0.66 H, H-2), 7.93 (s, 0.33 H, H-2), 12.56 (br s, 1 H, NH). MS (ES, positive mode): *m/z* = 589 $[M + 1]^+$.